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After geodynamics, what?

THE earth sciences depend very largely on international collaboration, so it is no surprise that there has been a succession of projects aimed at nurturing the free flow of data and ideas across national boundaries. International Polar Years, and the International Geophysical Year of 1957 led in the 1960s to the Upper Mantle Project and in the 1970s to the Geodynamics Project, which closes at the end of this year. Questions are inevitably being asked now about a possible successor to these programmes, and in Zurich last week, under the aegis of the International Union of Geological Sciences (IUGS), a wide variety of earth scientists gathered to review the state of their specialisations and to mull over a proposal for collaboration in the 1980s.

What does a low-budget international scientific programme achieve? In some respects very little. It offers no research funds. Most scientists in their national context are unlikely ever to read the rationale behind projects and even less are they likely to make a major adjustment in their research to take account of the projects stated aims. And in many, though not all, countries the availability of funds for the support of research is likely to be tied much more closely to the excellence of the investigator or the relevance of the proposed work to national needs than it is to the context of an international programme. Many of the remarkable discoveries in the earth sciences of the past twenty years have been made with little or no direct assistance from the prevailing project of the time. On the other hand, international projects can concentrate on two aspects of science which national systems are often not competent to assist—indeed sometimes seem positively to hinder: the bringing together of people from a wide variety of national and multi-disciplinary backgrounds, and the removal of blockages in making data easily accessible regardless of its national origin. On this basis alone the modest costs of a small secretariat, travel funds and data centres seem amply justified. Since all branches of science at all times profit greatly from this sort of assistance it is unfortunate that international unions have to convene working groups, write long reports and propose grandiose projects simply to maintain the rudiments of international and interdisciplinary scientific exchange.

Be that as it may, with the Geodynamics Project running out in ten months' time there is a pressing need for a new programme to be announced. Four years ago it seemed possible that the International Union of Geodesy and Geophysics (IUGG) might opt completely out of a further inter-union programme (the totally illogical way in which the geologists and geophysicists have separate unions and entirely different organisational machinery within them surely represents one interdisciplinary blockage which ICSU could do well to eliminate). In the past year and a half, however, IUGG has buried the hatchet and working groups convened by Dr H. Illies of Hamburg (IUGS) and Dr C. Kisslinger (IUGG) have now brought forward a joint proposal for the next ten years. But even if the respective

unions in their different ways accept the present document, it will not be until the summer of 1980 that final approval can be obtained from ICSU's general assembly, so there is bound to be a hiatus between one project and the next.

It is possible to identify three distinct threads of argument in discussions of this document. The first recognises that scientific advance is to a degree independent of international projects so simply reckons that the next ten years will build on what has already been learnt, though will doubtless contain some surprises—will in effect be a continuation of the International Geodynamics Project. The second believes that to be politically acceptable it will not be possible simply to say 'more of the same'—a new title will be necessary and new foci of attention will be called for. The Illies-Kissinger document carefully pulls together these two threads; the central theme of the proposed new programme would be 'the current state, dynamics, origin and evolution of the lithosphere, with special attention to the continental lithosphere'. (The lithosphere is the strong outermost shell of the Earth, of depth up to 150 kilometres beneath continents.) The programme proposes a change of focus and yet is a logical consequence of what has gone before.

The third line of thinking, however, says that earth scientists must respond to changing political climates, most notably in two areas: first, global resources, hazards and the maintenance of the environment; second, the need of the developing world for strengthened scientific and technological bases.

This geopolitical point of view has been put forward most forcefully by Professor L. Nicolaysen of the University of the Witwatersrand, Johannesburg. He recognises that the Illies-Kisslinger document does mention these matters, but would have them occupy a much more prominent position in project plans.

There are some fairly good administrative and bureaucratic reasons why this proposal can be resisted. It raises all sorts of questions that will offend countries' sensibilities, bring in new agencies, straddle difficult industry/academic frontiers, call for larger expenditure and so on. Quite so. But it is time earth scientists faced up to the uneasy barriers that exist between pure and applied research, and it is time they recognised the unique role they can play in the developing world. Maybe they should look, for a start, at the success of the International Centre for Theoretical Physics at Trieste in nurturing developing world scientists, and should wonder whether the earth scientists might evolve a similar project.

It would be a mistake simply to avoid these issues as being too political or too complex. The unions, whilst accepting the present document as a sound basis on which to proceed scientifically, should grasp the opportunity they have to bring together a highly fragmented range of disciplines, to take greater steps to bring basic and applied science together and to start serious thinking about responsibilities in the developing world. □



US boosts military support of campus research

Defense Department support for basic science is returning to pre-Mansfield Amendment days. **David Dickson** reports

AFTER a gap of seven years, the US Department of Defense has re-established an office to co-ordinate its efforts in basic research and to stimulate contacts with university research groups.

This move, which was recommended in a report on the department's basic research released by the Office of Science and Technology Policy in Washington last week, reflects an effort backed by President Carter to restore military support for university research to the levels of the late 1960s. Two factors have brought this about: renewed pressure for technological innovation—with requirements ranging from laser weapons to high-speed integrated circuits—within the Department of Defense; and the continued squeeze on other sources of support for university research.

The clearest evidence of the new trend is provided in the budget request submitted by the President to Congress last month. This asked for \$436 million for basic research obligations in the Department of Defense in the fiscal year 1980, almost 10% of the total basic research budget. If the request is accepted by Congress, this would mean an average increase for Defense Department basic research in the two Carter budgets (covering the period 1978–80) of 18.3%, considerably higher than for any other agency, and compared to an average increase of 11.4% for the National Institutes of Health, and 10.5% for the National Science Foundation.

Military support for basic research reached its peak in the late 1960s, when the efforts of university scientists in fields such as nuclear weapons were

supplemented by those recruited to support US efforts in Vietnam. Reaction against the conduct of the war, however, and the use of science in areas such as chemical weapons or counter-insurgency techniques, led to a wave of protests about military involvement on university campuses.

The protests became focused in an amendment made by Senator Mansfield to the Defense Department appropriations in 1970. Seen by some as merely a "good housekeeping" move, but by others as reflecting broader concerns about military involvement with science, the amendment stated that funds would only be provided to research projects bearing a "direct and apparent relationship" to a specific military function or operation.

Although revised a year later to require merely a "potential relationship"—a criterion which is still in effect—the Mansfield amendment contributed to a rapid shrinkage in Defense Department support for basic science, which in real terms was almost halved between 1967 and 1975.

In its report, the OSTP panel, which was chaired by Dr J. K. Galt of Sandia Laboratories and included representatives of universities, industry and other government agencies, states its belief that the current level of funding has reduced the utilisation of basic research in the Department of Defense "below a minimally acceptable level". It "welcomes and applauds" recent attempts to reverse the previous decline in research funding.

The panel says that such research is important to the department for three main reasons: to fill gaps in knowledge, to provide a source of new concepts,

and to keep up with technological developments in other parts of the world. "Growth for 10 years at a rate similar to the rate of decline of the past 10 years is indicated", it says. The OSTP panel places a special emphasis on the potential contribution of university scientists in single-mindedly pursuing long-term goals rather than performing highly-specified research. The universities are a "logical" place to expect original and startling new ideas, it says.

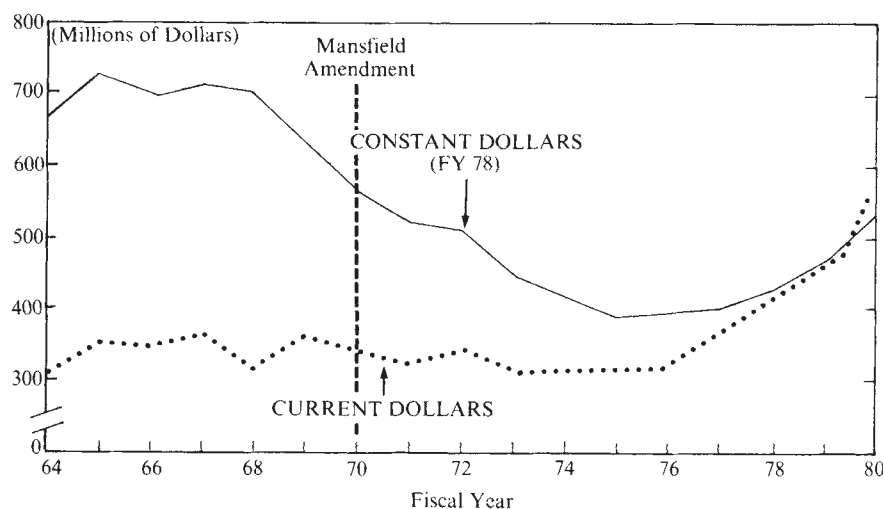
This emphasis on supporting university research is already reflected in the department's budget. Between 1976 and 1978, for example, while Defense Department support for in-house basic research was kept constant (in current dollars), support for basic research in universities increased by almost 60%. In 1976, universities carried out 33% of the department's basic research; by 1978 the proportion had increased to 40%.

There have also been shifts in the areas of research receiving support. From the US Air Force, for example, there has been a decline in support for nuclear physics or atmospheric research, and an increase in materials science and engineering. Mathematics, however, and particularly the computer sciences continue to receive good support.

So far there has been little opposition from the academic community to the re-emerging importance of Defense Department research funds. A few re-echo the concerns of the late 1960s. "The trend is part of an overall growth of military spending and militarism in this country that I consider very unhealthy for science in its broader context—it's like saying that wars are good for surgery", says Dr Charles Schwarz, professor of physics at the University of California in Berkeley.

And a group of scientists in Boston have recently recommended that basic research be removed from the Department of Defense budget, where they say it has a "high probability of military applicability". They have suggested that the majority of the funds be transferred to a National Technology Foundation, which would stimulate R&D in areas of technology where private industry is not yet willing to undertake the work.

But most scientists are taking a more pragmatic line. They point out that, unlike the more applied research sponsored by the Defense Department in university-associated laboratories—such as MIT's Lincoln Laboratory—the basic research it supports is unclassified. And it also has spin-offs in other areas. □



Basic research spending by the Department of Defense, 1964–1980. (Source DoD/OSTP)

DNA risks: fears calmed, but doubts remain

EXPERIMENTS carried out by research workers at the US National Institutes of Health have provided little evidence to support fears that bacteria carrying recombinant plasmids or phages containing a eukaryotic viral genome could themselves become producers of virus particles.

However, the research workers have shown that free viral DNA is capable of producing infection in mice when injected under the skin, and that infection can also be caused by recombinant phages in which the viral DNA occurs twice in succession. They have also raised the possibility, now receiving further study, that the recombinant molecules could present a hazard by transforming cells into a tumorigenic state.

These results have emerged from a series of experiments carried out at the top-level containment facilities of Fort Detrick in Maryland as part of a programme to assess the potential risks posed by the use of recombinant DNA techniques.

A team of research workers from the National Institute of Allergy and Infectious Diseases at NIH, headed by Dr Wallace P. Rowe and Dr Malcolm A. Martin, conducted two series of

experiments. One involved the molecular cloning of polyoma virus in a disabled *E. coli* plasmid vector system, and the other involved studying recombinants formed between a lambda phage vector and polyoma DNA.

In the first series of experiments, the research workers found that although all the recombinants studied contained the complete, potentially infectious viral DNA, in no case did either the recombinant plasmid, or live *E. coli* containing the plasmid, cause polyoma infection in mice.

In the second series, two types of recombinant phage were found to have formed, the most common containing a single copy of the polyoma DNA, but a second type containing two copies of the DNA linked head-to-tail in tandem. The phages containing the single copies was not infectious in mice, either when given directly, or when used to infect concentrated suspensions of *E. coli* from which the DNA was extracted and administered to the mice.

However, the phages containing the tandem inserts—theoretically more likely to cause infection since polyoma genomes could be generated by intramolecular recombination—did indeed prove to be infectious in two of five

mice inoculated with the phage DNA, and in two of five injected with DNA prepared from *E. coli* infected with the phage.

According to the research workers, whose findings are about to be published in *Science*, the latter result indicates that the possible cloning of head-to-tail inserts should be taken into consideration in carrying out risk assessment analysis of the cloning of DNA, although they say that such an occurrence is highly unlikely to occur, particularly in shot-gun experiments.

The research workers say that similar results on the low infectivity of cloned genomes can be predicted for other members of the *Papovaviridae* family of viruses—including the SV40 virus—as well as most other DNA viruses, containing larger and more complex genes.

However, they draw attention to a potential hazard that might be caused by the ability of bacteria containing virus DNA recombinants to induce tumours. This hypothesis is based on the ability of polyoma DNA to induce tumours in young hamsters by transforming cells; tests of any potential hazard are now being investigated. □

David Dickson

Science gets new champion on Capitol Hill

It is unusual to find a US Congressman demanding a strong, centralised state planning mechanism, a device usually associated with the nation's ideological rivals. Just such a mechanism, however, is being proposed by a member of the House of Representatives whose voice on science policy matters will be frequently heard over the next two years: Representative George Brown of California.

First elected to Congress in 1953, Mr Brown has been chosen to chair the science, research and technology subcommittee of the House science and technology committee. The subcommittee's responsibilities will be expanded by incorporating the domestic and international scientific planning committee; and the result will be to make it broadly responsible for overseeing the nation's research efforts and science policy.

Mr Brown is unashamedly partisan. "Some people talk of science as the handmaiden of society; I want to see science as a leader in our national culture", he said in an interview with *Nature* last week.

He is critical of a "laissez faire" attitude to research and development, where national concerns merely adjust the broad parameters of programmes

rather than their more precise dynamics. The US, he says, lacks "a co-ordinated national approach to giving both direction and resources to the scientific community".

With the general pressure on research budgets, funding innovations must concentrate on the reorganisation, rather than the expansion, of resources, he says.

If this sounds a bit vague, then Mr Brown can point to his recent experience in helping steer through Congress legislation to set up co-ordinated programmes of climate and earthquake research—efforts which have earned him widespread respect in the scientific community—as well as his involvement in agricultural and environmental research issues.

But not all agree with the primacy that he would allot to science. During last year's debate on the climate programme, for example, the OSTP successfully headed off an effort to make it lead agency for the programme, a task passed to the Department of Commerce's National Oceanographic and Atmospheric Administration.

Mr Brown remains convinced that OSTP should be at the top of the pile. Although welcoming the increased funds that the science adviser has



Congressman George Brown: "I want to see science as a leader in our culture."

helped secure for basic science, he claims that OSTP has not confronted to the intentions and the language of the legislation which set it up in 1976.

"We had hoped that the science adviser's office would give some coherence and strategic planning to science, and it has not done so, leaving quite a lot of unfulfilled expectations", he says.

At OSTP they see things slightly differently. "Some of these expectations were perhaps partly misplaced. We are not a kind of national clearing house on R & D", one official said last week.

Nevertheless mechanisms by which the nation's scientific efforts can be given "improved leadership" are high on Mr Brown's agenda. "The period of substantial growth experienced by science a decade ago has been extremely valuable; but it is now time to do some stocktaking". **David Dickson**

France attacks UN research on Mediterranean

Mediterranean countries are to slow cooperative research on pollution, writes **Robert Walgate** from Geneva

FRANCE, assisted by Spain and Italy, has succeeded in cutting three major projects from the United Nations Environment Programme's research effort on pollution in the Mediterranean. Research has been halted on pollutants in the open waters of the Mediterranean (as opposed to the coasts), on transfer of pollutants from the air to the water, and on modelling the Mediterranean ecosystem.

Last week's meeting in Geneva of the 18 countries which signed the 1975 Barcelona convention on Mediterranean pollution was dominated by money problems, in particular shifting payment for the Mediterranean action plan from UNEP—where responsibility has lain until now—to the states themselves. France, as the largest of the 18 countries, was concerned that its contribution should be well spent, and so devoted considerable efforts to analysing the research plans of UNEP's Regional Seas director, the Yugoslav Stjepan Keckes.

Keckes had developed a 13-part programme of research in the Mediterranean known as 'Med Pol' I to XIII. The first seven deal with coastal waters, and were approved by the original Barcelona meeting. It is Med Pol VIII to XIII which were added later and deal largely with open waters that were challenged last week.

The total research costs involved were relatively small—around US \$1 million a year, compared with an estimated cost of some \$5 billion for treating presently untreated Mediterranean sewage effluent so some matter of principle appeared to be at stake. If the countries were serious about dealing with Mediterranean pollution, why should they be bothered by the one part per thousand of the costs that came in the form of research?

The question mark hanging over the whole meeting was precisely what principle was at stake. According to the French delegation, the question was the quality of the research teams that UNEP had put together.

Keckes had, as a matter of principle, drawn together the expertise of existing government - sponsored laboratories

around the Mediterranean coast until he had some 83 participating labs in 16 countries. These varied greatly in quality, but he took the view that governments would respect the conclusions of their own labs more than those of externally imposed teams of 'experts', however good.

Further, by receiving advice and financial support from UNEP, these laboratories could be brought up to an international standard. So far, UNEP has spent some \$1.4 million on equipping these labs, mostly with American equipment (which an independent panel adjudged to be the best).

By Keckes's own admission, not all these laboratories have come up to scratch. In one southern Mediterranean state, for example, UNEP equipment languishes in a filthy laboratory without supplies of water or electricity, in spite of continued assurances that the necessary facilities will be supplied. On the other hand, neighbouring states are superior to some northern countries.

Keckes is undaunted by these setbacks, seeing the treatment of the Mediterranean and the improvement of developing country science in a very long perspective. But at Geneva the French delegation took a different view. They argued that the UNEP team could only cope effectively with local, coastal sources, and that the more complex issues of open-water pollution, air transport and ecological monitoring were beyond them. France already has programmes in these areas, said the delegation, and would make its information open to UNEP.

Keckes, however, thinks that the principle at stake may have been different—and closer to a question of the ownership of sensitive data. In the past he has found French officials "unforthcoming" about pollution of the Rhône river, which is one of the three major pollution sources of the Mediterranean (the others are the rivers Po—Italy—and Ebro—Spain), and he suspects that the same will be true of central Mediterranean data. This data is essential to an assessment of the long-term effects of pollutants, par-

ticularly cadmium and mercury and polychlorinated hydrocarbons (from pesticides).

Further, the air—whose circulation over the Mediterranean is little understood—may be one of the main sources of these pollutants. According to Keckes "the input of airborne pollutants may turn out to be the major unknown parameter needed to assess the state of pollution in the Mediterranean basin". Measurements in the Baltic have shown that between 10% and 90% of heavy metal pollution comes from the air, and in the Adriatic it has been observed that there is some 300 times more heavy metal in the top metre of the sea than in deeper layers. Polychlorinated hydrocarbons affect the reproductive cycle of fish, and so could have a long-term effect on Mediterranean fish-stocks—a matter of more interest to southern nations with a comparatively greater reliance on fisheries, than to industrialised northern states. Nonetheless, the air-sea research programme has been cut.

The French view is that not only is the Mediterranean team of poor quality to undertake such vital work, but the necessary instrumentation and methodology is not yet available to make an accurate assessment. Keckes admits that measurements so far are only accurate to an order of magnitude, but believes that it is necessary for the whole programme to proceed in parallel, so that each part can learn from the others. He hopes that the deleted research can be revived in UNEP's long-term programme for the Mediterranean, which will be agreed in 1981.

The Regional Seas team is not discouraged. On the contrary they had been led to expect far worse. Research is only part of the Mediterranean Action Plan, which includes 'priority action' (inexplicably including development of alternative energy sources) and the 'Blue plan' for coordinated environmental management and development of Mediterranean industry. The balance between these programmes has been maintained.

In the final analysis, the budget for the Mediterranean Action Plan for 1979 and 1980 was put at \$6.4 million. Of this, \$3.3 million will come from the Mediterranean states and \$1.6 million from UNEP. Within this the total budget for Med Pol for two years will be some \$880,000. The rest will be provided by other UN agencies such as the World Health Organisation and the United Nations Development Programme for parts of the programme related to their own interests. □



Soviets soften on psychiatry?

REPORTS from Moscow suggest that, for the first time, the Soviet psychiatric establishment is willing to engage in cautious dialogue with dissidents over alleged cases of political abuse of psychiatry.

Since its foundation in January 1977, the unofficial Moscow "Working commission for investigating the use of psychiatry for political purposes" (an adjunct of the Helsinki monitoring group) has been publishing regular reports of such cases. In Bulletin 13 (November, 1978), it reported the case of Yurii Zakharovich Valov, hospitalised apparently for having made repeated protests to the media against what he saw as unfair dismissal from his job in an engineering works. The diagnosis was not revealed; however, it was known that Valov had been pre-

scribed heavy medication—including insulin and chlorpromazine.

The latest bulletin, number 14 (January 1979), takes up the case again. According to Soviet practice, Valov's case was to be reviewed by a medical commission on 7 December. As well as the regular establishment doctors, a representative of the working commission was invited to be present—Dr Aleksandr Voloshanovich, a psychiatrist, who has been acting as a consultant for the working commission since last April.

The official report on the Valov case stated that Valov was in need of "biological treatment, psychotherapy, and subsequent socio-psychiatric measures". The exact grounds for this recommendation are not entirely clear though "personality disorders" have been mentioned.

Dr Voloshanovich agreed with the need for psychotherapy and "socio-psychiatric measures" (whatever they may be), but he denied that hospitalisation and the use of neuroleptics were indicated.

This is by no means the first case where extreme psychiatric measures were employed, on political grounds, against a person suffering from a mild psychological disorder. The invitation to Dr Voloshanovich to sit in on the case may well reflect a double approach to the working commission—a desire for cautious dialogue combined with a wish to obtain at least a partial confirmation of the official verdict.

Vera Rich



Dr Aleksandr Voloshanovich: asked to advise

Shortage of technologists in USSR

INTEGRATION of research and industry is a fundamental tenet of Soviet science policy. It is the rationale behind the establishment of a network of 'science centres' throughout the country, and the occasional Party censure of individual research bodies which neglect the industrial problems of their local area.

In recent months, however, official emphasis has been turning away from the role of the scientists in the 'scientific-technological revolution' to that of the technologists. Last July, Mr Brezhnev told the Plenum of the Central Committee that the shortage of middle-level managers and department heads in factories was disquieting, while in agriculture only 40% of such posts are filled.

Last week, *Pravda* took up the case of other organisations including scientific research institutes, where there is also a serious lack of middle-level staff. The problem seems to be a dual one:

the actual training of the required personnel and their deployment once they have qualified.

Technical college admissions, says *Pravda*, are constantly increasing (almost 1.5 million new enrolments last autumn). Nevertheless, it comments, "training has to be formulated taking into account long-range forecasts of the development of science and industry", which suggests that certain courses need re-designing and/or upgrading.

At the same time, the deployment of the present work-force of middle-level technologists—the "sergeants of industry"—is far from at its best.

The *Pravda* editorial (a traditional way of launching a new efficiency drive), calls for the relevant Party and Trade Union Committees, and the All-Union and Republic Ministries of Higher Education, to "analyse and eliminate" these defects.

Vera Rich

British scientists help Chinese set up new university

THE UK is to help China set up and run an English-speaking technical university. The idea for such a collaboration was first raised last November when a high-level delegation from the Chinese Academy of Sciences visited the UK. British officials showing the delegation around scientific establishments were taken by surprise by the proposal, which was announced towards the end of the visit. It was not clear, according to John Deverill of the Royal Society, whether the Chinese decided to propose the cooperation on the spur of the moment or whether they had planned it before their visit.

The university is to be established at Chengtu, the capital city of Szechuan, a province of central China covering 6% of the country's land area and containing 10% of its population. Chengtu Technical College, which has 2,800 students, 1,190 academic staff, and five departments is to be expanded to include 5,000–6,000 undergraduates, 1,500 postgraduates and 13 departments and given university status. The vice-chancellor will probably be Mr Ma Shih-tu, vice-president of the Chengtu branch of the Chinese Academy of Sciences.

The key UK bodies involved in the operation will be the Royal Society and the British Council, though no one is yet sure what it all entails. "The Royal Society is not set up to start universities in other people's countries", Mr Deverill says, but he thinks it could be arranged for academics to spend some time—from one to three years—at Chengtu helping to set up new departments and start new courses. The British Council will probably help establish English courses and arrange for English teachers to spend time there. The administration and funding of the university, however, are likely to be left to the Chinese. A delegation of British officials and academics now visiting China is assessing more precisely how the UK can help at Chengtu.

Chengtu will be unique as the only exclusively English-speaking university in China. The Chinese Academy of Sciences runs two other partially English-speaking universities at Hang-chou and Hofei, and one partially Japanese-speaking university at Harbin. The Chinese Ministry of Education used to run French and German-speaking universities in Shanghai. There is a possibility that both these could be restarted with French and German help.

Judy Redfearn

news in brief

Profit plummets after nuclear problems: Siemens, the West German transnational electrical company, has had a first quarter slowing of its sales, orders and profits because of difficulties with its subsidiary, Kraftwerk-Union, a nuclear contracting firm. Losses in KWU caused by problems in Iran, Brazil and a partial freeze on nuclear construction at home have offset an 8% increase in new orders for the rest of the Siemens group. Failure to authorise new power stations is "driving West Germany to catastrophe", said Dr Bernhard Plettner, Siemens chairman. As a way out, Siemens is trying to win a contract in Argentina to build a second nuclear plant in addition to a KWU facility in operation there since 1975. But as part of the deal, the Argentine government is demanding additional facilities to enable it to produce plutonium. The Bonn government faced severe criticism from the US for making this kind of arrangement with Brazil last year.



Swiss to vote on reactor safety: Radical new licensing measures for reactor safety to be inserted into the Swiss constitution will be voted on by the electorate on 18 February. The referendum includes: proposals for autonomous regional approval within a 30 km radius of all proposed sites; accident compensation without limits; 25-year operating concessions for both plants and the associated storage of waste; requirements that all catastrophe precautions be made public; cessation of construction or operation if at any time safety guarantees are withdrawn; and a bridging rule that will require existing plants to re-apply for approval under the new rules within three years. The result of a five-year effort by a coalition of Swiss anti-nuclear groups, the campaign got boosts from a successful site occupation in 1975 at Kaiser Augsp near Basle and the emergence of a strong women's contingent opposed to nuclear power. Swiss authorities believe that if the vote goes in favour of the proposals, three plants—at Leibstadt, Kaiseraugst, and Graben, where local opposition is particularly strong—will be blocked. The bridging rule could mean that existing plants at Beznau, Mühleberg (pictured above) and the brand new 920MW plant built by the West German firm KWU at Goesgen could be closed.

Energy conservation month: October 1979 has been designated International Energy Conservation Month by the International Energy Agency (IEA). The 19 member states of the IEA are organising a variety of events to take place during October with the aim of increasing public awareness of the need for energy conservation. Several international conferences are being organised including one on the use of urban waste heat in Tokyo last October, one on energy management to be held in Birmingham, UK on 9-11 October, one on the energy conservation policies in several countries to be held in Sweden and one on the use of solar-heated water to be held in Spain.

In its national programme, the UK is laying particular emphasis on energy management, an area in which it feels it has gained some expertise in recent years. During October it will also be relaunching the "save-it" campaign for 1979-80 at a cost of £2 million and publishing the report of the Advisory Council on Energy Conservation.

Spain will promote the month by organising a bicycle

day' and in Switzerland local authorities will be able to measure heat losses from buildings by means of aerial photographs. In general, the Mediterranean members of IEA such as Italy, Spain and Greece are hoping to demonstrate the uses and importance of solar power while some of the colder countries, for example, Denmark and Canada are laying more emphasis on conservation in buildings.

Aid to Vietnam: Vietnam's scientists, known to be desperately short of scientific equipment, are to be helped by a British charity. Medical and Scientific Aid for Vietnam has collected £8,000 to buy equipment for biologists in Vietnam. A spokesman said the money was raised in a year. Of the £8,000, £2,500 was provided by another charity, War on Want. The money has been used to buy gel electrophoresis and column chromatography equipment for work on protein separation; a spectrophotometer for routine analysis; and some general laboratory glassware. These items will help equip one of the country's newest laboratories—that of biochemistry and food chemistry in the Vietnam Scientific Research Centre in Ho Chi Minh City (formerly Saigon). The laboratory is doing agricultural research and scientists will be investigating disease resistance in rice as well as looking at cheap sources of nitrogenous fertiliser.

Bombay magnetic observatory threatened: Construction of India's largest fertiliser complex at Thal-Vaishet threatens the Colaba-Alibag magnetic observatory 5 km away. It is feared that magnetic observations taken over a continuous span of 133 years will be disrupted by electrical interference from the plant. Dr Stuart Malin of the International Geological Survey described Colaba-Alibag as "probably the most valuable magnetic observatory in Asia", but added that "the fertiliser factory is undoubtedly more important to India than the observatory". The factory has been opposed by the Save Bombay Committee, a group concerned with the pollution risk to 37,000 people 10 km downwind from the site. The committee will take the case to the Bombay High Court.

Bangladeshi centre for appropriate technology: Bangladesh is to set up a National Centre for Appropriate Technology at Bangladesh University of Engineering and Technology (BUET) that will cost \$4 million and will be completed in two phases: the first in 1980 and the second in 1985.

The broad objective of the centre is to identify, develop and adapt technologies appropriate to the basic needs of the people of Bangladesh. Its studies are to be sponsored by government, autonomous bodies, private organisations and international agencies. It will provide training and research facilities and will maintain contact with similar organisations throughout the world.

Its major task will be to co-ordinate activities between scientific institutions and organisations in Bangladesh. Agriculture is the first area it will be considering. It is proposing to set up regional centres at the Agricultural University, the Academy for Rural Development, the Agricultural Institute, the Rice Research Institute and at three engineering colleges. It will monitor the diffusion of technology to the regional centres. Networks of institutions are also to be set up for industry, communications and energy with the new national centre as the focus.

The centre will also work on the design and fabrication of new products and the modification and adaptation of imported goods. Its aim is to provide government with policy alternatives on the import, adoption and adaptation of foreign technology.

Flaws in GMAG's guidelines

'We urge that time be allowed . . . before any firm decisions about major changes in the approach to categorisation are made' — **Royal Society**

● A statement issued by the Council of the Royal Society and prepared by an ad hoc group chaired by Professor W. F. Bodmer. Other members were: Sir David Evans, Professor D. C. Phillips, Dr M. G. P. Stoker, Sir Frederick Warner, and Sir Robert Williams.

WE welcome the attempt of the Genetic Manipulation Advisory Group's paper (*Nature*, 9 November, page 104) at a more rational approach to the categorisation of the experiments which come under its jurisdiction, using the framework of risk assessment. The chemical and nuclear industries have considerable experience of quantitative risk assessment, based on fault-tree analysis, and hazard and operability studies, backed by extensive data on reliability. In the longer term we believe that advantage should be taken of this experience and so we are requesting the Royal Society's recently established Risk Study Group on the Assessment and Perception of Risk, under the chairmanship of Sir Frederick Warner, to include the GMAG paper and the application of risk assessment techniques to genetic manipulation in its considerations.

Even in areas such as the chemical and nuclear industries, where extensive background data are available, there is often disagreement among experts. We believe that the present knowledge of the quantities needed for a quantitative risk assessment in the area of genetic manipulation is quite inadequate for this now to form a basis for categorisation of experiments. The absolute risk associated with any experiment involves the product of many probabilities (all, of course, less than one) some of which are known and known to be low, and so this risk may in many cases be estimated to be very low, even given our present incomplete knowledge. Categorisation of experiments, however, requires the estimation of relative risks, or ratios between probabilities which in most cases will be small, and, at the same time, quite imprecisely defined, and so we do not see how these ratios can yet be calculated with sufficient accuracy to be useful for categorisation.

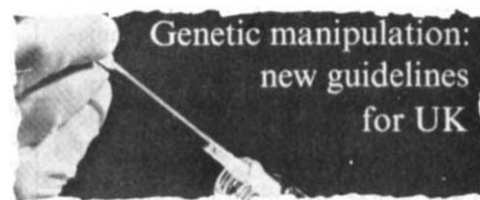
Particular areas of ignorance are the probabilities of human error and the real risks associated with the ingestion of the experimental product, namely the host carrying a recombinant vector. In the case, for example, of vaccine development such as for polio, which

probably involved much greater potential risks than most work with recombinant DNA, extensive data were collected on the *in vivo* effects of potential vaccines in animals and humans, on the basis of which the magnitude of the risks could be confidently assessed.

There is an urgent need for more comparable data on the recombinant DNA products and on their associated hosts and vectors, such as *E. coli* K12 and its mutants, phage lambda and other plasmid vectors. Until such data, and other relevant information required for quantitative risk assessment, are available we believe the approach suggested in the GMAG paper, and appropriate developments of it, should be used for studies of the existing categorisation, rather than for categorisation itself. If, for example, further testing fully supported the present evidence that *E. coli* K12 could not be converted into an epidemic pathogen by recombinant DNA techniques, then it might be possible to assign nearly all recombinant DNA experiments using normal *E. coli* K12 host-vector systems to category I, or at most category II.

The real risks, if any, associated with most experimental products of genetic manipulation are, as already pointed out, the most difficult to assess since they are all, so far, conjectural. While it is appropriate to encourage research in areas which might be thought relevant to the assessment of these conjectured risks, as in the GMAG paper, we believe that the discussion of such conjectured risks should be adequately documented by reference to the scientific literature and subject to comparable standards of scientific scrutiny.

The discussion in the GMAG paper falls far short of these standards. For example in describing risks (the section headed "The Conjectured Hazards") there is a progression from "conjectured" through "likely" and "potentially severe" to "possible" with no justification whatsoever. The mere existence of a list of conjectured risks introduces the notion of their probability, when some may even be impossible. In other contexts, risks of this sort have been classified, by consensus of those knowledgeable in the field, into (1) negligible, (2) possible but perhaps doubtful, and (3) definite though possibly small risks. It might be instructive to do the same with some of the conjectured risks that have been



Genetic manipulation:
new guidelines
for UK

attributed to work with recombinant DNA.

The difficulty in applying the suggested new approach to categorisation is illustrated by some of the chosen examples in the GMAG paper. "Self-cloning", for example of *E. coli* K12 genes into *E. coli* K12 host-vector systems, is assigned to category I and made the subject of extensive notification and detailed reporting to GMAG, whereas the Health and Safety Executive's definition of genetic manipulation, accepted as unchanged by GMAG, specifically excludes such experiments from its remit (through the use of the phrase "... incorporation into a host organism in which they do not naturally occur . . ."—our italics).

We deplore any extension of GMAG's remit, implicit or explicit, especially into areas where there is evidence that the risks are negligible and which form a significant part of experimental work in modern biology. Any such extension should be subject to very careful further consideration and justification and should not arise automatically from the adoption of a new procedure.

It is also not at all clear why experiments involving the expression of cholera toxin genes in *E. coli* K12 need to be performed under conditions which are considerably more stringent than those required for work with *Vibrio cholera* itself. We urge that any recommendation for a higher level of containment for recombinant DNA work with a pathogen, than for work with the pathogen itself, at equivalent doses, be carefully justified. In many cases involving, for example, work with certain animal viruses it is likely, as has often been pointed out before, that recombinant DNA experiments with parts of the viral genome will entail much less risk than work with the whole virus.

We believe it to be very important that GMAG consider its procedures carefully in relation to the new US National Institutes of Health (NIH) Guidelines which came into effect on 2 January 1979. These new guidelines were prepared after extensive open, documented and subsequently published consultation with many groups in the United States and abroad, including in particular Europe and the United Kingdom, especially through the European Molecular Biology Organisation. In the earlier considerations of the

problems posed by genetic manipulation, following the "Ashby" (Cmnd 5880) and "Williams" (Cmnd 6600) Reports, the approach followed independently by the United Kingdom was quite close to that followed in the United States and indeed at times in the forefront.

The new NIH Guidelines, which are being adopted in some form by most other countries that are advanced in this field in Europe and elsewhere differ, however, quite significantly not only from the new GMAG proposals, but also from some of its current practice. They differ, for example, with respect to self-cloning experiments and in the use, in general, of set categories for given types of experiments, rather than accumulated case law. A set list of categorisations obviates the need, once an institution is appropriately registered, for repeated referral back to a central authority before starting any particular experiment. Now that a significant amount of case law has accumulated, we urge GMAG to consider following this approach for an increasing proportion of experiments under its remit, and so simplify its activities and those of the people it serves, while in no way jeopardising recommended standards of safety.

We believe that in cases where GMAG's procedures lead to substantial differences in categorisation from those recommended by the NIH Guidelines, the difference must be carefully justified. A lack of comparability between procedures in the United Kingdom and elsewhere, especially in the United States, could seriously jeopardise this country's research and development efforts in this important area of work and in particular, threatens our contribution to extensive international co-operation in research in this field.

In this connection we would draw attention to the coordinating activities of COGENE, the Committee on genetic experimentation set up by the International Council of Scientific Unions. It should be noted that COGENE jointly with the Royal Society is organising an important meeting in April 1979 to bring together those concerned from many countries to consider these problems.

We have emphasised the need to obtain much more quantitative data to form the basis for a quantitative risk assessment. The pooling of data on risks with the US and other countries, as is done, for example, in the nuclear industry, would greatly extend the available data base needed for a proper quantitative risk assessment.

The timing of requests for responses to the GMAG paper, just before Christmas, and its earlier working paper in the summer, has been un-

Arithmetical perils

RECENTLY I gave my class in elementary haematology a midterm examination that included a 'free gift' question: "A patient has an erythrocyte count of 4.57 million per cubic millimetre and a blood haemoglobin level of 14.1 gm per decilitre. What is the mean corpuscular haemoglobin content?" The answer, in picograms per erythrocyte, routinely appears on computerised read-outs of blood analyses. Most of the class refused to attempt the question. I think they felt its intricacies were so mentally disturbing that to tackle it would interfere with their answering the rest of the examination.

Arithmetical problems also arise with residues in food. The Delaney Clause of the Food Additives Amendment is interpreted as prohibiting the presence in food of any added carcinogen at any level, no matter how low. Supporters of the clause often say that there is no "non-carcinogenic" level of any carcinogen. This "no-threshold" concept is sometimes arbitrarily extended to toxicological effects other than cancer. This controverts the essential basis of the science of toxicology that the dose determines the poison. As a result, per cent, parts per million and parts per billion (ppb) merge hazily into each other in the minds of many people. I wonder how many mathematicians would willingly drink a glass of water if they were told it contained 30 ppb of arsenic? Yet the human body normally contains 40 to 300 ppb.

There was a protest about the arsenic content of California wines in one of our local magazines, with the usual charges about the malfeasance of those who would poison for profit. The authors vividly described the agonies resulting from arsenic over-dosage, without mentioning the quantities involved. They also hinted darkly at a University of California cover-up of the analytical results on wine. I pointed out that the offending wines had a range of 5 to 116 ppb of arsenic and that marine fish contain 2,000 to 8,000 ppb. It was no use; the authors stuck by their smoking guns, and talked about arsenic and cancer, and the rights of consumers to be informed of "the complete known and suspected contents of all food, beverages, drugs, water and



Thomas H. Jukes

air." I hope that they stop by the health food store for some dried kelp, and ask for information on its arsenic content.

The arithmetic of selenium has stumped the finest minds in the FDA for about 20 years. It seems that they think selenium is a carcinogen. It came into initial prominence in the 1930s as a teratogen in farm animals that were fed wheat from seleniferous soils in the Dakotas. In 1957, it was found at the National Institutes of Health, and simultaneously by our group at Lederle Laboratories, that selenium was an essential nutrient, and within a few months, selenium deficiency in farm animals was reported from many parts of the world, especially in New Zealand, but also in the USA, despite the excess in the Dakotas. Mindful of the Delaney Clause, the FDA refused to permit addition of selenium, as salts, to animal feeds. Of course, they could not stop the use of fishmeal as a source of selenium. A little arithmetic would have helped: about 0.1 to 0.3 part per million of selenium in the diet is essential, Delaney or no Delaney, and 5 to 10 ppm is toxic.

The emotions aroused by arithmetical amblyopia reach a peak in disputes over fluorine. A Pittsburgh judge has just acceded to impassioned pleas that fluoridation of the local water supply be halted. The water contained 0.4 ppm of fluoride before fluoridation, and the procedure that was stopped raised this to 1.0 ppm. Apparently the added 0.6 ppm was ruled to be deadly, for some non-arithmetical reason. Incidentally, sea-water contains 1.4 ppm and supports a flourishing fauna, including creatures which have splendid teeth.

fortunate and the period allowed for a response very short. It is not clear why such a major change in approach should be treated with such urgency. We urge that time be allowed to collect more risk data, to assess the risk assess-

ment approach in relation to current categorisation, and to assess the impact of the new NIH Guidelines, before any firm decisions about major changes in the approach to categorisation are made. □

correspondence

Researchers and the SRC

SIR,—We are a group of non-tenured research faculty who have been working for the University of Sussex for periods up to and exceeding ten years, during which time the university has benefited both from our skills and reputation and from the capital funds attracted by our research.

We have read with interest the recent articles in *Nature* which discussed the problems of people in our situation and would like readers to be aware of our existence as a group and of our position on the issues. We feel that the problem—while certainly a national problem—can, in fact, be solved by local action at university level: the issue is not one concerning redundancy payments, but that the university can, and should, provide permanent tenured employment for those of us who have demonstrated our proficiency and usefulness in the past.

The discussion in *Nature* has so far been concerned with researchers who derived their support from the MRC but there are many who have been supported for long periods by the Science Research Council. The SRC seems to support fundamental research in this country on the hypocritical assumption that it is the universities and their tenured staff who are carrying out the research. In fact, because of the increasing complexity of research projects and the increasing demands on the time of tenured faculty, it seems that no university group can perform any really ground-breaking research without the help of some full-time, post-doctoral research personnel. Under present conditions these research personnel are almost always non-tenured. In spite of the essential role played by these non-tenured researchers in doing the actual research the SRC maintains a policy of not supporting non-tenured research workers beyond a certain age.

In the past there was enough turnover in university and national laboratory personnel so that most non-tenured research workers eventually found a post. Also, the University Grants Committee used to provide funds to allow research projects to be "taken over" by universities but this no longer occurs. Many SRC officials, and perhaps the Council itself, still seem to feel that the universities are under an obligation to give permanent employment to research workers even though the universities claim this is impossible to do.

Even when they are in a position to make appointments the universities are not living up to their responsibilities. The majority of recent new university posts have been limited to the first few points on the lecturer's scale, thereby restricting them to people about thirty or younger. One of us was told by a professor who had just made an appointment, that he did not think any university would ever offer him a lectureship because of his seniority—referring both to his age and abilities.

On a less personal level, the university here has refused to give any undertaking, in negotiations with the AUT, to show some preference to older people in temporary positions when and if new posts become available.

It is clear that in many cases the SRC is aware of the current situation. One of our members is currently supported under a research grant which contains a special condition that requires the university to employ only that particular individual as the researcher on that project, thus explicitly recognising his essential role. The SRC must however face the fact that at the moment scientific research in this country is conducted on an unconscionable basis, with untenured research personnel being treated like serfs in a feudal society, rather than the workers providing the intellectual capital which is essential to the future of a society which prides itself on its democratic traditions. The present organisation of research is basically inefficient and unstable and cannot be continued much longer: the conditions of employment of non-tenured research workers would not be tolerated in any other sector of our society.

Present SRC policies include: age restrictions on appointments to SRC establishments; age restrictions on the non-tenured research personnel supported by SRC grants; attempts to force the universities to "take over" research contracts and personnel while the UGC refuses to provide the resources for such "take overs"; and restriction of the right to apply for SRC research grants to tenured members of university faculties. These are making an already difficult situation incalculably more damaging than it has to be.

There is evidently a need for basic research if Britain is to maintain any sort of competitive position in the developed world. The only way we can compete in international markets is by making maximum use of the skills and creative abilities of our people not by discarding those of us who have mastered and perfected some of the most sophisticated skills necessary for complex modern technologies.

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Seismic prediction at Pozzuoli

SIR.—Tazieff (8 September 1977, page 96) makes accusations that I deliberately put about wrong data in 1970 concerning warning signs that an eruption could occur in the vicinity of the city of Pozzuoli. This is not the first time he has made this statement and I have already answered him in a technical note presented to the Pontanian Academy in 1973, a copy of which I sent to him. I can state that it is completely untrue that I was the source of bulletins or news of an impending eruption in Campi Flegrei. Indeed to the best of my knowledge no

such news was spread at all.

Perhaps I may go further. Tazieff speaks of false claims of a "conspicuous temperature rise of the Solfatara's fumaroles"; but in 1970 I wrote "geothermal measurements at the Solfatara have not shown up to June 9 (the date I corrected proofs), any sensible variation in temperature". Tazieff further speaks of false claims of extremely shallow earthquakes, claiming his team disproved their existence. A paper by I. Guerra *et al.* in *Quaderni della Ricerca Scientifica* No. 82 (1972) reproduces the seismograms from these quakes and mentions a "superficiality of foci". Finally Tazieff says that the vertical ground movement or brady-seism was no faster than usually noticed over the centuries as opposed to claims that the ground had been pushed up one metre in a "very short while". Readers can judge for themselves by looking at Casertano *et al.* (*Nature* 264, 161–164; 1976) whether the motion was average—in my reckoning it went up to 60–70 times average.

During my involvement in these matters I acted according to my moral duties without any external pressure. Only after the order to move out of tottering houses (not the city, as Tazieff declares) could I rest peacefully, and even then of course hardly with much pleasure at seeing poor people evicted. In fact eviction saved lives, maybe as many as fifty because buildings fell down in 1970 and 1972. Tazieff indeed supported a motion on 18 March 1970 which excluded the possibility of people rehhabiting old crumbling houses.

Very briefly, my involvement was this. I first reported the inversion of the Phlegrean brady-seism to the authorities on 12 February 1970, mentioning a ground upheaval of about 80 cm in little more than a year. I warned of the possibility of seismic phenomena and that even the continuation of the brady-seism might unsettle the buildings in the area. At the first shock (1 March 1970) a decree evacuating tottering buildings was issued, as a precaution. 650 shocks were recorded up to 16 November 1970, six felt in Pozzuoli itself at up to IV on the Mercalli scale. Three died in November from falling masonry and rocks. In my letter of 12 February I had foreseen a seismic, not an eruptive phenomenon.

For the sake of brevity I have only refuted a part of Tazieff's gratuitous statements: it would be possible, quoting published papers to amass much more scientific criticism. Tazieff states arbitrary conclusions in order to give value to the serious charge of a supposed participation in a "construction operation". He gives no direct proof, nor does any exist, while the indirect ones he reports are wrong or deliberately altered. Therefore all of his statements are nothing but products of fancy.

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news and views

Chromosomal aberrations at very low radiation dose rates

from John R. K. Savage

ONE effective way of determining whether an individual has been exposed to ionising radiation is to look for an increase in structural abnormalities in the chromosomes of their white blood cells after the cells have been induced to divide in the laboratory. The exposure to radiation produces chromosome damage which shows up at mitosis as structurally abnormal chromosomes in the resulting daughter cells. In certain cases it is even possible to calculate the dose of radiation received (Dolphin, Lloyd & Purrott *NRPB Report R13*; 1973; Dolphin, Lloyd & Purrott *Health Phys.* **25**, 7; 1973). (Chromosome-type aberrations following radiation fall into two main categories. Asymmetrical, which always result in an acentric fragment and are readily scorable with conventional staining. These are also termed 'unstable', as few cells carrying them can survive cell division. Symmetrical aberrations, on the other hand, do not result in acentric fragments and relatively few are scorable without recourse to special staining methods. Such aberrations are termed balanced because they do not pose any mechanical problem or lead to genetic loss at cell division and so the changed chromosomes can be passed on to the cell progeny (Savage *J. med. Genet.* **13**, 103; 1976)). This method has chiefly been used to detect acute radiation exposure, usually at levels above the permitted maximum, although the data are often difficult to interpret in cases of accident or environmental exposure where the ideal methodological conditions are impossible to obtain. In this issue of *Nature*, however (page 531), H. J. Evans and colleagues have shown that the technique can be successfully used to demonstrate a cumulative effect of very low doses of radiation over 10 years in nuclear-dockyard workers who have been exposed to doses well below the internationally agreed maximum permissible level of 5 rem per year.

At very low doses, or with very long exposure times, when a long period is required to accumulate a significant dose, many of the radiobiological problems (such as dose rate or dose fractionation) associated with the effects of large acute doses on aberration yield disappear. Uniform exposure throughout the body also becomes less important as circulation, and the mixing of the lymphocyte pool, will tend towards greater dose uniformity in the sampled population. Similarly, differences in the mode of formation of aberrations by radiation of different qualities (which shows up as the degree of curvature in the dose-response curve) are reduced, and all categories of aberration increase linearly with dose.

Cumulative doses over long periods have problems of their own however. For example, after an acute dose, there is a gradual reduction in asymmetrical (unstable) aberrations with time *in vivo*, as a result of the loss of aged cells, accompanied by an influx of 'normal' cells by division of lymphocyte precursors. These 'normal' cells will of course also include the progeny of those cells with symmetrical aberrations. The half-life of unstable cells in circulating blood is around 3 years (Dolphin *et al. op. cit.*), so with prolonged exposure there is a continuous loss of damaged cells at the same time as new damage is being generated.

The effects of long-term exposure are complicated by the fact that the exposed subject is ageing. If there is any change in the spontaneous frequency of aberrations with increasing age (and there is evidence for this in man), or if there is any change in intrinsic radio sensitivity (very little within the age range considered here (Evans in *Chromosomes and Cancer* (ed. German) 191, Wiley, New York, 1974), the observed aberration yield may well show an increase with accumulated dose since larger doses will be received by older subjects, provided they continue at risk.

Such complications must therefore be

eliminated before a positive dose effect can be established. This has been done by careful statistical analysis in Evans *et al.*'s paper, and a significant effect for unstable aberrations results. Aneuploidy, and chromatid-type aberrations, neither of which are likely to be the result of radiation exposure (in this experiment at least) did not show any such dependence upon dose, although both showed positive increase with age.

The absence of a significant effect for symmetrical (stable) aberrations is puzzling, and cannot be entirely explained away by the difficulties of detection, since one assumes that scoring efficiency for this class is constant at each sampling time. Furthermore, such aberrations are not expected to be selected against by the same mechanisms which eliminate unstable forms, so one might have anticipated accumulation at a faster rate. The application of modern banding techniques now enables these types to be scored with high efficiency, so such questions as relative selection could be solved. Certainly such forms persist for up to 60 years in dermal fibroblasts (a very slow turnover cell population) following therapeutic radiation (Savage & Bigger in *Mutagen-induced Chromosome Damage in Man*, 155, University Press, Edinburgh, 1978), though some recent experiments in Syrian hamster skin (D. Couzin, unpublished) suggest that there may be a gradual elimination even of symmetrical forms with time *in vivo*.

The observed rate of aberration accumulation (which equals the dose-response curve in this case) is probably some function of the turnover rate of the cell population. Experiments with chronic γ -irradiation in plants (Evans & Sparrow *op. cit.*; Sax *Science* **112**, 332; 1950; Sparrow & Evans *Brookhaven Symp. Biol.* **14**, 76; 1961) have shown that aberrations can reach an equilibrium frequency, the level of which is influenced by both dose rate and cell kinetics. Possibly it is the very slow turnover of lymphocytes that allows an accumulation to be recorded.

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In which case the picture might be quite different for other tissues in these workers, particularly in those with a fast cell turnover. This must be borne in mind when extrapolation is made.

It is important to note that the corrected results represent a finite 'population' trend over several years. Similar findings within an individual, sampled on successive occasions, would be almost impossible. The magnitude of a Poisson confidence interval at this sort of aberration frequency is not generally appreciated. Even the four-fold increase above background found here would not be detected without a phenomenal amount of scoring, as the authors point out.

The principal significance of the findings lies in the demonstration that effects at these very low dose rates can be found if care is exercised in the analysis to eliminate confounding factors.

The increase reported here has been

established for visible unstable types which are invariably cell lethal. We may infer that similar increases probably occur in stable types and in some changes too small to be detected by conventional methods. It is these transmissible types where any genetic significance must be sought. However, their importance depends not only on the nature of the changes but where in the genome they occur and also in what cells they are found. For example, very gross chromosomal changes, orders of magnitude above those found here persist in the fibroblasts of human skin for many years following therapy without impairment of other functions or any problems for the individual. As pointed out above the rate of accumulation is likely to vary between cell systems and one will want to know what happens in other tissues, such as testis, before a real evaluation of any possible hazard can be made.

□

hydrothermal systems have shown how water brought into rock by downward percolation over a wide catchment, is exhaled at the thermal centre. Corless has shown that the abundance of many elements in the oceans may in fact be controlled by hydrothermal processes and not by continental run-off as has been previously thought. These experimental data have allowed a whole new perception of the interdependence of hydrosphere and ocean crust.

Sato derived a series of elegant models to show how hot, metalliferous, acidic brines could be either more or less dense than sea-water, this depending on the balance between temperature and the amount of dissolved solids. Interaction of such brines with the effectively infinite reservoir of sea-water would result in rapid cooling and dilution. Such conditions would destabilise the chloride complexes causing the metals to precipitate. The lower density fluids would disperse, precipitating their metals over a wide zone. Such a process explains well the pronounced metal enrichments in sediments around all the mid-oceanic ridges. It could indeed account for the metal content of manganese nodules. The denser fluids would remain on the sea-floor and flow down into the nearest topographic low, there to precipitate their metals. In many cases the site of exhalation may itself be a depression and the metals would accumulate above their feeder.

Three sources for the sulphur have been proposed—the fluid itself, inorganically reduced sea-water and organically reduced sea-water. The abundance of carbonaceous sediments associated with exhalative ore deposits and the remarkable discoveries of an aphotic biota around hot-springs on the ocean-floor near the Galapagos Islands has enhanced the case for an organic reduction of sea-water. Sulphur isotope studies have usually indicated an inorganic source.

Such then was the agreement between experimental and field data that discovery of sulphides on the sea-floor was only a matter of time. Numerous examples of metalliferous sediments, of ferruginous and manganiferous crusts and indeed of active, but rather cool, springs have been reported in recent years. However the CYAMEX team have found the first sulphides. The technical problems of navigating and sampling in an oceanic rift are stupendous. The fact that the crew recognised the oddity of these rocks and sampled them is a credit to them and we can only look forward eagerly to results of their further investigations at the site.

But what exactly have they found? It would appear from their descriptions that they have, not a massive sulphide lens as such, but the deposits around

Sulphide ore deposits

from J. P. N. Bedham

It is rare that the exponents of so observational a science as geology can have the satisfaction of saying 'we told you so'; rarer still does the prediction turn out to be lucrative. The discovery of 'massive' sulphide deposits on the East Pacific sea-floor by the CYAMEX team fulfills just such a prediction. To be sure we have had the stupendous reserves of the Red Sea brines for some time, but, despite the denials of Cronan and others, there always remained the lingering doubt that in so small an ocean, so close to land and so near to major salt deposits something odd may have happened. What was needed to dispel this doubt and to satisfy the financiers of deep sea research programmes that they may be on to a winner has now been found.

What were the predictions based on? It has been accepted for some time now that ophiolite complexes are the only parts of oceanic crust to have escaped destruction in subduction zones. Many of these complexes contain copper-zinc-iron sulphide ores. Indeed one—the Troodos in Cyprus—has been mined for so long that there is debate as to whether the metal gave its name to the island or *vice versa*. The Troodos ores occur in lenses (1 to 10 million tons) capped by ochres (ferruginous muds), cherts and umbers (manganiferous muds). They have long been recognised as chemical sedi-

ments. The massive sulphide lenses are underlain by disseminated mineralisation in highly altered host-rocks. These are always pillow lavas and are usually the earliest in the sequence—that is, they were formed at the axis of a spreading-centre. Their alteration was caused by a hydrous fluid at temperatures ranging up to 500 °C. Less intense hydrothermal alteration has been recognised throughout the upper 3 km of the ophiolite. Such are the field observations supporting an origin of the ores at the spreading-centre by precipitation from an exhaled fluid. However, the problems of sources of the fluid, metals and sulphur and of the mode of precipitation required an experimental approach.

Lister has shown that sea-water can interact with lava to generate an ideal potential ore-fluid. The interaction cools and hydrolyses the lava and the fluid becomes hotter, more concentrated and acidic. Such a fluid can selectively leach base-metals from rocks and transport them in solution as chloride complexes. The rate of cooling of newly generated oceanic crust requires that the predominant heat loss is by convective circulation of sea-water. Spooner and others have shown convincingly from isotopic studies that the water which altered ophiolite piles was essentially sea-water. Both Lister and Spooner, by separate approaches, have argued that the data require many rock-volumes of water to have traversed the altered strata. Models of

the vents of hydrothermal springs. They have shown that sulphides can be formed directly on the sea-floor—that is, that sulphur is an integral part of the system and not a later, but necessary, additive. The spires of sulphides and silica lie directly on pillow lavas and the fluids that were exhaled from these vents have deposited the bulk of their load elsewhere. Where? Were the fluids heavier than sea-water, in which case we can expect the imminent discovery of a genuine massive sulphide lens nearby; or lighter, their metals dispersed? These are the questions that must now be investigated. The chances of finding a massive sulphide lens are probably low—the system appears to have been oxidised too soon and too close to its vents, and many of its metals may never have been precipitated.

Finally, what of the economic

implications? The recovery of massive sulphides from the sea-floor would seem to be economically remote with no shortages in terrestrial reserves of copper and zinc. Potentially cheap extraction of metalliferous brines offers a far better prospect. It is to this that the mining houses must turn their eyes. Is this discovery a complete fluke or could there really be a ribbon of sulphides along the ridges? Mid-oceanic ridges are generally far too exposed to the physical and chemical vagaries of the open oceans to have encouraged large scale, undisturbed, reduced environments for the common preservation of massive sulphides. Back arc basins, such as the Japan or Philippine seas offer the same magmatic and hydrothermal processes, but in a far more protected environment. It is in these that a discovery of major sulphide deposits is more likely. □

The *recA* gene product

from B. A. Bridges

THE *recA*⁺ gene in *Escherichia coli* is involved in many cellular functions; in particular it is absolutely required for general recombination (as distinct from site-specific or illegitimate recombination). It is also required for a number of processes which have been suggested to be 'inducible' by DNA damage. The prototype *recA*⁺-dependent inducible process is induction of prophage λ and this is the only *recA*⁺-dependent process for which a repressor (λ immunity repressor) is known and characterised. For others, such as Weigle reactivation, filamentation or control of DNA degradation, the existence of a repressor remains hypothetical. One thing that is clear, however, is that the *recA*⁺ gene product is made in very large quantities following treatments (such as ultraviolet irradiation) which elicit prophage induction and other 'inducible' phenomena.

A recent breakthrough towards understanding the function of the *recA*⁺ gene product came with the observation that a proteolytic activity capable of cleaving λ repressor was present in extracts of cells known to contain large quantities of a mutant *recA* protein specified by an allele of *recA* called *tif* (Roberts *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 2283; 1977). Such cells constitutively express 'inducible' functions. Recently, the *recA* protein from *tif* cells has been further purified to chromatographic homogeneity and an ATP-dependent proteolytic activity specific for λ repressor has been shown to be associated

with it (Roberts *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 4714; 1978). *RecA*⁺ protein possesses only about 20–25% of the proteolytic activity associated with the variant protein from *tif* cells.

The discovery of λ repressor inactivating activity associated with *recA*⁺ protein has tended to focus attention on its possible role in controlling 'inducible' functions to the relative neglect of its role in genetic recombination. Yet the earliest characterisation of the protein, which was only subsequently found to be the product of the *recA*⁺ gene revealed that it bound to single-stranded DNA (Gudas & Pardee *J. molec. Biol.* **101**, 459; 1976) and there have been many observations suggesting that *recA*⁺ protein at its normal relatively low level must interact directly with DNA during both recombination and repair, without any requirement for synthesis of large amounts such as might follow induction.

Such a direct role of *recA*⁺ protein in DNA metabolism has now been established. Following the report (Ogawa, *Cold Spring Harbor Symp. quant. Biol.* **43**, in press) that *recA*⁺ protein hydrolyses ATP in the presence of single-stranded DNA, Weinstock, McEntee and Lehman (*Proc. natn. Acad. Sci.* **76**, 126; 1979) have shown that *recA*⁺ protein purified to near homogeneity catalyses an ATP-dependent renaturation of complementary single strands of DNA. The relative activities at different temperatures of the protein from a cold-sensitive *recA* mutant confirm that this activity is an intrinsic function of the *recA*⁺ protein. Thus *recA*⁺ protein is a 'windase' or

'annealase' and as such can be readily envisaged to have a specific primary role both in recombination and in the repair of damaged DNA.

In a parallel investigation following independent purification, Shibata, Das Gupta, Cunningham and Radding (*Proc. natn. Acad. Sci. U.S.A.* in the press) have pursued the matter one step further and shown that the *recA*⁺ protein can catalyse the ATP-dependent pairing of superhelical DNA and homologous single-stranded fragments to form D loops. Thus single-strand ends are aggressive and can invade homologous superhelical DNA in the presence of *recA*⁺ protein; in its absence, such a reaction occurs only at high temperature. D loops are known to be a substrate for some endonucleases (including the *recBC* DNase). One can therefore begin to see how recombination may be initiated and the way now lies open for exploring the whole process *in vitro*.

In view of the involvement of the *recA*⁺ gene in controlling degradation of damaged DNA and its possible involvement in allowing error-prone base insertion opposite a damaged template strand, it would also be interesting to investigate the effect of *recA*⁺ protein *in vitro*, on say, exonuclease V activity and the 3'→5' (proof-reading) exonuclease activity of DNA polymerase III. Three recombination-proficient alleles of the *recA*⁺ gene, namely *tif*, *recA*⁺ and *lexB* all yield a gene product with similar 'annealase' activity although they differ markedly in expression of inducible functions.

The data so far are consistent with *recA*⁺ protein's being rather complex in its activities, apparently showing specific proteolytic activity, DNA-dependent ATPase activity and ATP-dependent 'annealase' activity. Moreover, it seems also to control its own induction and synthesis. Such complex activity in a simple tetramer of subunit molecular weight 40,000 is not inconceivable but neither is it common.

Because normal levels of *recA*⁺ protein are insufficient to cause derepression of prophage λ one may well imagine that many phenomena depending on constitutive levels involve its acting as an annealase, for example normal recombination, post-replication recombinational repair, mutation in unreplicated DNA and repair of single-strand breaks produced by ionising radiation (this is not to say that raised levels of *recA*⁺ protein might not enhance these processes). Phenomena that occur only with elevated induced levels of *recA*⁺ protein could involve either annealase activity (for example, control of DNA degradation, multiple crossovers during Hfr x F⁻ crosses (Lloyd *J. Bact.* **134**, 929; 1978)) or re-

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pressor cleavage (λ induction for example) or possibly both. In some cases (Weigle reactivation, filamentation) the mechanism is still obscure.

There is still, however, a nagging doubt about ascribing the proteolytic activity to the *recA*⁺ protein. As Roberts *et al.* state, they cannot exclude the possibility that a contaminating protease is present in their preparations even though they are chromatographically homogeneous. Consistent with this possibility is the observation that bacteria containing constitutive high levels of *recA*⁺ protein due to the presence of the controlling mutation *spr* do not show constitutive λ induction nor do crude extracts possess high ability to inactivate λ repressor (Roberts *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 4714; 1978). Several workers have suggested that the *recA*⁺ protein requires a cofactor to be active in prophage induction. It is not totally inconceivable that such a cofactor might be the protease and that it is present in greater amounts in *tif* mutants (although such speculation does present its own difficulties). Thus although the evidence that *recA*⁺ protein is both a protease and a DNA annealase may be sufficient to sustain the idea as a working hypothesis, more definitive evidence must be sought and the possibility of further revelations of the functions of this remarkable protein cannot be excluded. □

Chamber crystals in the stratosphere

from Brian A. Thrush

ONE aspect of stratospheric chemistry which has been largely overlooked in the recent flurry of activity has been the role of the aerosol particles. These form the Junge layer in the lower stratosphere and have their peak density at an altitude of about 18 km. Although these particles, which have sizes in the range 0.05–1 μm , number only about one per cm^3 , the amount of material they contain is comparable with the amounts of important trace materials in the lower stratosphere, such as the nitrogen oxides. If these species reacted efficiently with the aerosol particles, this process could be significant to the chemistry of the stratosphere. Chemical analysis shows this aerosol to contain ammonium sulphate, accompanied by persulphate and perhaps chloride, but recently Farlow *et al.* (*J. geophys. Res.* **83**, 6207–11; 1978) have tentatively identified two nitrosyl sulphuric acids, NOHSO_4 and NOHS_2O_7 by electron diffraction studies of aerosol particles collected by U-2 aircraft.

Poles apart?

from Clive Lloyd

THERE is still much to be learned about the mitotic apparatus, especially the nature of spindle organisers. Such microtubule-organising centres (MTOCs) are believed to exist at each spindle pole, where, by nucleating the assembly of microtubules, they help construct half-spindles along which sister chromatids are guided to opposite poles. *In vitro* studies confirm that microtubules can self-assemble, but the value of MTOCs is that they localise assembly and so help control planes of division important for shaping tissues. The most familiar MTOC is associated with the centriole which is a highly structured organelle resembling a vestigial flagellum. It is noteworthy, though, that amorphous pericentriolar material would seem to be the spindle organiser in *in vitro* polymerisation studies on Chinese hamster ovary cells so, if nothing else, the centriole provides a convenient signpost for such associated material (Gould & Borisy *J. Cell Biol.* **73**, 601; 1977).

Definite pole bodies are to be seen in algae and fungi, but in higher plants we are confronted with the problem that such structures are not visualised in ultrastructural studies of dividing cells. This has forced the alternative view that accumulated endoplasmic reticulum seen at the poles could, by sequestering calcium, precipitate localised formation of microtubules (Hepler in *Mechanisms & Control of Cell Division* (eds Rost & Gifford) 1977), although it is admittedly difficult to see how, in the absence of a template, the number, pattern and precise location of microtubules could be controlled.

However, a recent study on the effect of a herbicide on 3T3 fibroblasts rekindles interest in presumptive spindle organisers since the drug is well known to produce mitotic abnormalities in plants. Oliver *et al.*

(*Expl Cell Res.* **116**, 229; 1978), showed that chloro-isopropyl N-phenyl carbamate (CIPC) causes the formation of multinucleate cells by inducing multipolar mitotic spindles. *In vitro* polymerisation studies indicated that CIPC, unlike colchicine, did not interfere with tubulin assembly and so it was concluded that the herbicide had affected spindle MTOCs rather than microtubules *per se*. This report on animal cells echoes the earlier study of Hepler and Jackson (*J. Cell Sci.* **5**, 727; 1969) on dividing nuclei in the endosperm of the African blood lily. Whereas colchicine caused microtubule disassembly, IPC did not, but with the latter treatment multipolar spindles were formed. However, although microtubules were seen to radiate from some central locus, electron microscopy failed to give shape to the spindle pole. Following this, Coss & Pickett-Heaps (*J. Cell Biol.* **63**, 84; 1974) used IPC to examine division in the green alga, *Oedogonium cardiacum*. They, too, found that multinucleate cells were formed, suggesting that the herbicide increased the number of MTOCs. No such bodies could be identified in thin sections but with high voltage electron microscopy on thick sections the picture was quite different, for morphologically distinctive spindle pole bodies were revealed in both normal and drug-treated mitoses. This family of reactions on representatives of animal cells, higher plants and algae indicates that some divisible MTOC is being affected by the herbicides but it remains to be determined whether endoplasmic reticulum, as opposed to some other associated, perhaps amorphous, nucleation site, is being perturbed in higher plant cells. □

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The stratosphere is very dry, containing only a few parts per million of water, and it is interesting to note that NOHSO_4 is very stable under such conditions. It is familiar to chemists as forming the 'chamber crystals' which appear in the lead chamber process for making sulphuric acid. They are formed from sulphur dioxide and nitrogen dioxide (NO_2) when insufficient water is present. The authors point out that if this compound comprised 20% of the sulphate in stratospheric aerosol then this would contain about one third of the amount of nitric oxide (NO) which is present at these altitudes.

Because the nitrogen oxides NO and NO_2 catalytically destroy ozone in the stratosphere, any process which affects their concentrations is potentially important. In fact, neither the measured ratio of NO and NO_2 to nitric acid (HONO_2) nor the failure to detect pernitric acid (HO_2NO_2) in the lower stratosphere agree well with predictions based on known homogeneous chemical reactions. Reactions involving aerosol particles may be implicated here.

In the stratosphere, these nitrogen compounds come mainly from nitrous oxide (N_2O) derived from denitrification processes and could therefore be

enhanced by increased use of nitrate fertilisers. Some nitrogen oxides are directly injected into the stratosphere from the exhausts of high-flying aircraft. For this reason it is tempting to see the formation of 'chamber crystals' as a means by which the stratosphere can simultaneously purge itself of two pollutants, but any such contribution will be confined to the lower atmosphere (the troposphere). Settling of stratospheric aerosol would not provide a significant process for removing nitrogen oxides from the stratosphere, by comparison with rain out of nitric acid. Furthermore, the increase in stratospheric aerosol in recent years is generally attributed to volcanic eruptions such as Agung in 1963 rather than to the increased burning of fossil fuels because of the efficiency with which tropospheric processes remove sulphur oxides. □

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As an illustration of the general character of the woodcuts which so profusely adorn this volume [Cassell's Natural History, volume II] we have selected one of an interesting animal which has been a great puzzle to the systematic zoologist. "The Binturong (*Arctitis binturong*) is a curious little animal of a black colour, with a white border to its ears; it has a large head and turned-up nose; its tail is immensely long, thick and tapering, and which is very remarkable it is prehensile, like that of a new world monkey. It is quite nocturnal, solitary and arboreal in its habits. Its howl is loud. It is placed by Mr Parker among the group of the civets." From *Nature* 19, 13 Feb. 345; 1879.

The spiral structure of galaxies

from Bernard Jones and Scott Tremaine

ONE of the most vexing problems in theoretical astrophysics has been the explanation of the beautiful spiral patterns seen in many galaxies. It is easy enough to produce spiral structure in both galaxies and coffee cups: because they rotate differentially (the inner parts revolve faster than the outer parts), an irregular gas cloud or a dribble of cream is rapidly sheared into a spiral feature which looks very similar to a galactic spiral arm. The difficulty is that by now most galaxies have undergone 50–100 revolutions, so that any primordial spiral arms would have been wound up beyond recognition.

The most likely resolution of the winding problem for differentially rotating galaxies is that the spiral arms are not material objects, but waves of gravitational potential which propagate around the galaxy. As the interstellar gas passes through the wave it is shocked and forms bright blue stars which we see as a spiral arm; the spiral arm appears narrow because the lifetime of the stars is short compared to the time the wave needs to propagate around the galaxy. This part of the theory is comparatively well understood, but understanding the origin and

persistence of the underlying wave pattern in the gravitational potential has been surprisingly difficult.

Most of the work in the past 15 years has been directed towards explaining the wave as a normal mode of the galaxy, like the oscillations of a drumhead. One difficulty with this picture is that the oscillations are damped by the very shocks that make the spiral arms visible, and the damping time for the normal mode is much less than a typical galactic lifetime. Another problem is that observations show that spiral arms are always oriented in the same sense: their convex sides face in the direction of galactic rotation. This clue tells us that galaxies are not time-reversal invariant,

because time-reversal would change the direction of galactic rotation. A neutral oscillation is time-reversal invariant and a theory based on neutral oscillations therefore cannot explain this observation.

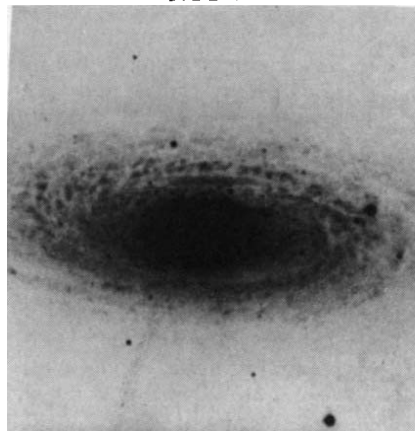
The difficulties with the simple normal mode theory for the origin of spiral structure have stimulated many important dynamical studies of galaxies. What has emerged is the realisation that differentially rotating galaxies, like the diaphragm of a drum, need to be continually excited to maintain their vibrations. A galaxy that is not excited in some way loses its grand spiral structure very quickly. Two kinds of "drumstick" have been proposed: either a satellite galaxy or a central bar-like structure such as is seen in many galaxies could provide a perturbing potential which would excite strong spiral waves.

J. Kormendy and C. Norman are the first astronomers to confront these theoretical ideas with a systematic and careful consideration of observational data; (*Astrophys. J.*, in the press). They collected pictures of all spiral galaxies whose rotation curves were known. Their sample contained 69 galaxies, of which 15 had to be discarded either because they appeared edge-on and the spiral pattern was invisible or they were too irregular to classify. Of the remaining 54 galaxies, 25 had bars and 8 had close satellite galaxies and as the theory would predict, the presence of such objects was always accompanied by well-defined large scale spiral arms.

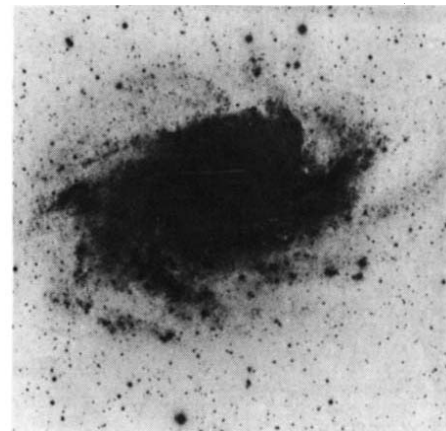
The most interesting galaxies are the remaining 21; they apparently have no known way of sustaining a spiral wave. Kormendy and Norman found that in every case, either the spiral arms were short and ill-defined, or the galaxy rotated uniformly in the region where the arms were prominent.

Retrospectively, it is easy to understand this result. In the case where the region occupied by the spiral arms rotates uniformly, there is no winding problem and the spiral structure is

NGC2841



M33



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relatively easy to maintain. In a region of strong differential rotation, the vibrations are sheared into small scale spiral structure. The difficulty in spiral structure theory has always been to explain the galaxies which rotate differentially yet show well organised long spiral arms. What Kormendy and Norman have argued is that it is just these galaxies that are always accompanied by a bar or a companion.

Two figures taken from their paper serve to illustrate their general conclusion. NGC 2841 is an archetype of those galaxies that have neither bar nor companion and have no coherent spiral pattern. This is to be contrasted with M33 which has neither bar nor companion, yet has a clearly identifiable spiral design contained within the region of the galaxy where there is almost solid-body rotation. The region of solid-body rotation in NGC 2841 is much smaller in relation to the size of the visible disk. What we may be seeing now is the emergence of a galaxy classification scheme that can be tied directly to galactic dynamics. □

T-cell regulation

from Rod Langman

DISCUSSIONS at the most recent Armand Hammer Workshop* centred on suppression and suppressor factors elaborated by T lymphocytes, Ir-gene effects and H-2 restriction, and on the question of idiotype determinants on T-cell factors and T-cell receptors. An impressive array of data was closely scrutinised; however, there was little discussion of how this data could be interpreted in terms of the current theories of immunology. The calm of hindsight has revealed some concepts and details that must be settled before we are in a position to manipulate the immune system rationally.

The network concept of an immune system has captured the imagination of many immunologists, and while associative recognition theories of immune regulation are less popular, they seem to fit many of the facts better. What, then, are the critical differences between these two viewpoints? First, the network demands a system of cell-cell activation and inhibition interactions that are balanced, and which exist independent of antigen. Associative recognition theories require cell-cell interactions to occur only by way of antigen bridges which signal activation, leaving suppression with a modulating role during antigen-induced activation. Second, the network demands that sup-

pression be paramount in the discrimination between self and non-self (which leads to unresponsiveness towards self antigens) while in the associative antigen recognition theories, unresponsiveness to self is a case of T-cell deletion that involves mechanisms other than suppression.

With these distinctions drawn between the two conceptual approaches to immunology, what experiments are most likely to resolve the differences? On the question of self-tolerance the radiation chimaeras will probably prove the most informative. For example, consider a mouse of strain A which has been thymectomised, irradiated, reconstituted with fetal liver (AT × FL, for short) and grafted with an (A × B)_{F1} thymus. When this mouse is immunologically mature, say 8–12 weeks post restoration, removal of the *F1* thymus graft will (1) remove the tolerated B antigen, and (2) prohibit any further T-cell maturation. If the suppressionists and network theorists are right then reactivity to B should be readily recovered; whereas, if the opposition is correct, reactivity to B will not be recoverable.

Another aspect of the self and non-self distinction deals with the phenomenon of H-2 restriction. Reduced to its simplest components in the cytotoxic T lymphocytes (CTL), overall specificity of effector cells is determined by two different antigen sets; one encoded within H-2K or H-2D (restriction antigens), the other encoded elsewhere, either by viruses or other genes such as H-Y (X antigens). The restriction (R) antigens are essentially within the 'self'-domain, whereas the X antigens are within the 'non-self'-domain. There was some discussion as to whether CTL recognised R and X antigens separately or only a new antigenic determinant (NAD) formed by the complexing of R and X antigens. However, M. Cohn (Salk Institute) argued that restriction specificities (that is, anti-K/D) are selected in the absence of X (that is, non-self) antigens (as shown by Bevan and Zinkernagel's early chimaera experiments) and therefore NAD's cannot be the only class of antigens recognisable by CTL. Thus, it seems difficult to avoid having two antigen recognition sites on the T cell, one for self (anti-R), the other for non-self (anti-X), and from this it follows that each site must carry different idiotype determinants. However, if each CTL carries two different idiotype determinants, then each CTL will have conflicting inputs from within the idotype-regulated network. In short, the best available interpretation of H-2 restriction is simply

incompatible with network theories. As a consequence, believers in the network insist that H-2 restriction must be a complicated form of suppression; but nobody has proposed a mechanism.

Despite the demise of the network there remains a body of data showing that idiotype recognition can be involved in the regulation of immune responses. Within the framework of associative recognition, idiotype recognition, and even idiotype specific regulation, can certainly occur, but it must be a second-order phenomenon, not primary as the network theorists believe.

Restrictive recognition can be readily incorporated into associative antigen recognition theories, but this opens the question as to the origin of the receptors for restricting and X antigens. There is a growing body of evidence that receptors on some classes of T cell have idiotype specificities which parallel those found on B-cell derived immunoglobulins, and these idiotypes are apparently controlled by genes mapping in *V_H*. Oddly enough, the immunoglobulin *V_H* controlled idiotypes usually require *V_L* for expression of the idotype, while in T cells there is no evidence for an L chain related to the Ig set (that is, κ or λ). The receptors bearing NP-idiotype isolated from T cells (K. Rajewsky, Köln), and the ARS and GAT-idiotypes found on T-suppressor factor (B. Benacerraf, Harvard University) have been serologically and chemically analysed and provide strong evidence that at least some T cells have receptors which carry idiotypes controlled by *V_H* genes. It is important to question the significance of these findings in terms of the role of these idotype-bearing T cells in regulating normal immune responses. One is left to ponder quietly the fact that it is only anti-idiotypic antisera that put *V_H*-determined idiotypes on T cells. This is especially worrisome in light of the known mutations which affect T-cell responses in an antigen-specific way; without exception, they are not *V_H* linked. Are the anti-idiotypic antisera leading us down the right or wrong pathway? Perhaps it is too soon to judge, but on purely theoretical and aesthetic grounds the T-cell receptor is unlikely to be encoded in the *V_H* locus; the H-2I(JEC) region offers intriguing possibilities.

The phenomenon of Ir genes, particularly those mapping to the H-2IA and H-2I(JEC) regions which show complementation, has continued to fascinate. If there is any consensus among Ir-gene speculators, the macrophage or antigen presentation system seems the most likely site of Ir-gene action. Perhaps the best support for the macrophage-Ir relationship came from experiments based on an antigen-

*An Armand Hammer Workshop on the problems of T-cell regulatory mechanisms was held at The Salk Institute, San Diego on 26–30 November, 1978.

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driven, macrophage and T-cell dependent, proliferative assay (PETLS), which showed that a mixture of two complementing non-responder macrophages were able to complement in the stimulation of F_1 T cells while of course the F_1 macrophage did stimulate (W. Paul, National Institutes of Health, Bethesda). This problem might be clarified by the use of two chimaeras. For example, if A and B are two complementing non-responder, the following two chimaeras would test complementation effects: (1) an $(A \times B)F_1$ is grafted with A and B thymuses separately so that F_1 macrophages present antigen to a mixture of A and B restricted cells; and (2) an $(A \times B)F_1$ mouse is irradiated and reconstituted with a 50:50 mixture of A + B cells which will then differentiate in an F_1 thymic epithelial environment so that each cell, T and macrophage, is genetically either A or B but is restricted to F_1 antigens. The results from these two chimaeras will distinguish between intracellular complementation and intercellular complementation.

Some Ir-1 gene phenomena are closely tied to restricting antigens, H-2K, IA and D, while others are linked to the H-2I(JEC) region which is not known to code for any restricting antigens. Although from the known genetics and structure of immunoglobulins it is expected that V_H and V_L should complement in generating unique antigen-binding specificities, it is rarely suggested that Ir-A and Ir-(JEC) gene complementation signify interactions of V_L - V_H type. However, a combination of serology and chemistry has shown that there are molecular, and possibly antigenic, complexes formed between gene products from the I-A and I-(JEC) regions (Uhr, Southwestern University; H. O. McDevitt, Stanford University). Another intriguing possibility is that I(JEC) encodes the V region of the T-cell receptor. Thus, after 15 years, we still seem to be grasping for a conceptual framework that converts the phenomena of Ir genes into commonsense understanding. In fact, this is a fair comment on T-cell regulation in general. $\square$

Confining current in tokamaks

from J. G. Cordey

THE poloidal magnetic field in a tokamak fusion reactor, which is essential for plasma confinement, is sustained by a toroidal current. In the present experiments this current is driven by an electric field which is produced inductively by arranging for the toroidal plasma to be the secondary of a transformer. This technique of generating the toroidal current is inherently pulsed and since it seems that for a reactor to be economic it will have to be run in a steady state mode, some alternative steady state method of generating the current will have to be found.

The first such scheme was put forward by Ohkawa (*Nucl. Fusion* **10**, 185; 1970), his suggestion was to inject a separate species of fast ions in the direction parallel to the field line so that these ions circulate around the torus and form a toroidal current. The strength of the current is proportional to the number of transits the fast ions make around the torus before becoming thermalised. This number is referred to as the stacking factor S and equals $\tau_s \bar{V}_t / 2\pi R$ where τ_s is the fast ion slowing down time, $\bar{V}_t$ is the mean velocity of the fast ions, R is the major radius of the torus. Thus the total fast

ion current is $I_0 S$ where I_0 is the injection current.

The electrons gain momentum from the fast ions through Coulomb collisions, so they also have a net drift velocity in the same direction as the fast ions and hence tend to reduce the total current. The expression for the net current which is obtained by balancing the forces acting on the electrons may be put in the form $J = I_0 S (1 - Z_t/Z_{eff})$ where S is the stacking factor, Z_t the charge number of the fast ions, and Z_{eff} the effective charge number of the plasma. Thus it would appear at first sight that unless the plasma charge and fast ion charge numbers are different there would be no net current. However, if the effects of the trapping of electrons in the toroidal field gradient and the non-Maxwellian nature of the electron distribution are taken into account, there is a net current, even when $Z_t = Z_{eff}$.

The first measurement of this beam-induced current was made on the Culham Levitron (Start *et al. Phys. Rev. Lett.* **40**, 1497; 1978). In this experiment a modulated beam of fast protons was injected into the device and the net circulating current was obtained from the voltage induced in a pickup coil which looped the plasma. The current was measured as a function of electron temperature and found

to differ significantly from the predictions of the simple theory. The discrepancy was due to the inadequacy of the theoretical model and a kinetic treatment (Cordey *et al. Culham report CLM-P547*; 1978) which took into account the non-Maxwellian nature of the electron distribution and electron-electron collisions was found to give much closer agreement. The implications of the new theory for tokamaks are fairly minor since the correction term is of the order $\bar{v}_t^2/v_e^2$ (where v_e is the thermal velocity of the electrons), which is usually small in tokamaks. The full expression for the beam-induced current in a tokamak including trapped electron effects (Connor & Cordey *Nucl. Fusion* **14**, 185; 1975) and the above correction

$$J = I_0 S \left\{ 1 - Z_t/Z_{eff} + 1.46 \epsilon^t \frac{Z_t}{Z_{eff}} A(Z_{eff}) - 1.2 \frac{\bar{v}_t^2}{v_e^2} \right\}$$

where $A(Z_{eff}) = 1.68$ for $Z_{eff} = 1$; 1.18 for $Z_{eff} = 4$ and 1 for $Z_{eff} = \infty$. There has been no clear identification of this current in a tokamak yet. However, sufficiently high neutral injection power is now available on several experiments and so one expects an observation of this current in the near future.

Another method of generating the toroidal current has recently been proposed by Fisch (*Phys. Rev. Lett.* **41**, 873; 1978). In this scheme a small group of high energy electrons are accelerated in a direction parallel to the field lines by high phase velocity r.f. waves. The frequency of the waves is near the lower hybrid frequency and they are launched from an "end fire waveguide" array, which directs the power flow parallel to the field lines. The waves transfer their momentum to the electrons through resonant particle damping and this drives a current in the plasma.

The power required to drive the current is calculated from the power dissipated by the group of resonant electrons through collisional drag on the remainder of the plasma. An effective r.f. resistivity $\eta_{r.f.}$ is then defined and this may be compared with the ohmic resistivity η_{ohmic} of the plasma. For equal currents the ratio of $\eta_{r.f.}/\eta_{ohmic} = 10 v_d^2/v_t^2$ where v_d is the drift velocity of the electrons in the ohmic case and v_t is the velocity of the resonant electrons in the r.f. case. Thus for low current levels (v_d small) the r.f. current dissipates more power than the ohmic current. At higher current levels, provided $v_t \gg v_e$ the r.f. current begins to dissipate less power than the ohmic current.

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Calculations are given for reactor parameters, and it is found that the power required to drive the current is approximately 3% of the total fusion power output and that only 1% of the area of the wall would be required for the wave guides.

Clearly an experimental demonstration of both the fast ion and r.f. schemes in a tokamak is required before any realistic assessment of the reactor potential of either of these schemes can be made.

□

Masses of exotic nuclei

from John Edgington

THE stable nuclei occupy a narrow strip on a plot of proton number Z versus neutron number N . Increase Z or N by a few units, and the resulting nuclei undergo β -decay; increase Z or N greatly and even this limited stability disappears, the excess nucleons being ejected singly or in clusters.

This fragility of all but a limited number of nuclei has to be explained, eventually, in terms of the individual nucleon-nucleon interactions. Given the complexity of many-body calculations, it is hardly surprising that for practical purposes, such as estimating nuclear binding energies and masses, simpler prescriptions are used. These 'semi-empirical' formulae are based on the liquid-drop model but include the main features of the individual interactions in an approximate way. The values of the numerical coefficients involved are adjusted to fit the masses of stable nuclei, and the most widely used of these formulae, the Garvey-Kelson relation (Garvey *et al.*, *Rev. Mod. Phys.* **41**, S1; 1969) also considers correlations between nuclei with closely similar values of Z and N .

The most stringent tests of these formulae occur, of course, for nuclei far from the narrow stability strip. Since these are very short-lived, some subtle experimental techniques are needed. A fine example is that reported from the LAMPF accelerator, Los Alamos, by a LASL/Northwestern University group (Seth *et al.*, *Phys. Rev. Lett.* **41**, 1589; 1978) who have measured the mass of ^{18}C . This exotic nuclide, with twice as many neutrons as protons is one of a class of nuclei having $N = Z + 6$ which seem to mark a significant boundary on the stability map of light nuclei. If N and Z are both odd, these nuclei are unstable to particle emission; if even, they appear to be stable. Like other neutron-rich nuclei, ^{18}C can be produced fairly readily in heavy ion collisions, or as a spallation product of heavy nuclei, but these violent processes are quite unsuited to precise mass measurements.

Seth and his colleagues have produced ^{18}C by the double charge

exchange (DCX) reaction $^{18}\text{O}(\pi^+, \pi^-)^{18}\text{C}$, using the energetic pion spectrometer at LAMPF and measuring the energy of both the incident π^- and the outgoing π^+ , to obtain the binding energy of ^{18}C by subtraction. The technical difficulties are great. There is a huge background of electrons from π^0 production and decay, but these are entirely eliminated by time-of-flight and Cerenkov counter techniques; also, the spectrometer's energy resolution is too poor for mass measurements, so a calibration reaction involving nuclei with known masses, $^{12}\text{C}(\pi^-, \pi^+)^{12}\text{Be}$, was also measured. The group had previously confirmed (LA-7457-PR Progress Report, Sept. 1978) that their equipment had the desired sensitivity, using the simpler reaction $^9\text{Be}(\pi^+, \pi^-)^9\text{C}$, since a target of natural beryllium metal poses fewer difficulties than the enriched ice target used for the ^{18}O DCX reaction.

Seth *et al.* compared their result for the mass excess of ^{18}C , 24.91 ± 0.15 MeV, with the Garvey-Kelson relation. Earlier predictions had suggested a somewhat lower binding energy, but a recalculation of the G-K prediction, using recent measurements of the ^{17}C and ^{19}N , yielded a mass excess of 25.04 ± 0.10 MeV, in excellent agreement.

It is worth stressing that Seth *et al.*'s measurement would not have been thought possible, even with technical ingenuity, two years ago when the cross sections for the DCX reactions (π^-, π^+) were thought to be exceedingly small. Conventional wisdom had it that reactions connecting analogue nuclear states were much more likely than those between non-analogue states. Roughly, analogue states are corresponding states in different nuclei which differ only in that one or more protons and neutrons have been interchanged, but no rearrangement of the nucleon orbitals has occurred. According to this opinion, any necessary orbital rearrangement would depress the cross section greatly. In 1977 another group working at LAMPF, B. M. Freedom's LASL/University of South Carolina/ANL collaboration, showed that the reaction $^{18}\text{O}(\pi^+, \pi^-)^{18}\text{Ne}$, connecting analogue states in the two nuclei, had a cross section only about twice as

large as that of $^{18}\text{O}(\pi^+, \pi^-)^{16}\text{Ne}$. The latter converts neutrons in a filled shell to protons occupying the next higher orbital, and so connects non-analogue states. Now, whereas it is always possible to find nuclei with excess neutrons on which the (π^+, π^-) reaction can connect analogue states with no orbital rearrangement, there are no stable nuclei (other than ^1H and ^3He) with excess protons; and so (π^-, π^+) reactions necessarily connect non-analogue states.

The results of Freedom's group (*Phys. Rev. Lett.* **38**, 149; 1977; *Phys. Lett.* **69A**, 55; 1977) greatly encouraged study of (π^-, π^+) reactions, since there is now seen to be no reason to suppose that their cross sections are unusually small. Even so, Seth *et al.* observed only about twenty events leading to the ground state of ^{18}C . They now plan to measure the mass of several more extremely neutron-rich nuclei, including ^{48}Ar which has $Z = 18$, $N = 30$; this will be reached by the reaction $^{48}\text{Ca}(\pi^-, \pi^+)^{48}\text{Ar}$. We may expect other such measurements in the near future, especially as the re-evaluated Garvey-Kelson relation now predicts greater stability against particle emission than before.

□

The current state of eukaryotic messenger RNA

from H. R. V. Arnstein

THE isolation of different messenger RNAs and their translation into specific proteins in a number of cell-free systems are now almost routine. Moreover, the nucleotide sequences of several messengers have been established. Given that the concept of mRNA emerged less than 20 years ago impressive progress has been made.

At a recent EMBO Workshop* the main topics were heterogeneous nuclear RNA (hnRNA) processing, hnRNA-protein complexes, free cytoplasmic messenger ribonucleoproteins (mRNPs), the cytoskeleton in relation to protein biosynthesis, polysomal mRNA-protein complexes and translational control.

The general picture which emerged was that the molecular aspects of transcription and the subsequent processing of the primary transcripts are known in outline, but there is still considerable scope for detailed studies of the enzymes involved and of the control of mRNA synthesis in eukaryotes. Moreover, almost nothing is

*Held at Hapert, The Netherlands on 27 November–1 December, 1978. The meeting was organised by H. Bloemendal and W. J. van Venrooij of the Biochemistry Department, University of Nijmegen.

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known about the transport of processed mRNA from the nucleus into the cytoplasm and there is uncertainty about the proteins which occur in association with mRNA precursors in the nucleus and with free and polysomal mRNA in the cytoplasm. These mRNA-protein complexes are a characteristic feature of eukaryotic cells, but their functional significance is obscure.

As discussed by J. E. Darnell (Rockefeller University) for adenovirus mRNA, capping of transcripts takes place very early, perhaps at the beginning of transcription. RNA polymerase reads past the site at which poly(A) is subsequently added and the primary transcript is cleaved endonucleolytically before addition of the 200-residue poly(A) tail. Methylation then takes place, followed by splicing to give the final processed transcript. The size of the primary transcripts of eukaryotic genes remains somewhat controversial with K. Scherrer (Cancer Institute, Lausanne) still favouring very large products. This question will ultimately be settled by comparisons of nucleotide sequences of genomic DNA, primary transcripts and processed messengers. The use of restriction endonucleases and DNA cloning has led to rapid progress as illustrated by the discussions of globin gene expression (R. Flavell, University of Amsterdam), the structure of late SV40 mRNAs (W. Fiers, University of Ghent), post-transcriptional processing of the yeast tyrosine tRNA, which contains a 14 nucleotide intron (J. S. Beckmann, Beth-Dagan, Israel), and mouse pre-mRNA structure (A. P. Ryskov, Inst. Molecular Biology, Moscow).

The complexity of heterogeneous nuclear RNA was illustrated by the isolation from nuclei of both 30-50S monomers and heterogeneous polymers, which differ in protein composition and resistance to ribonuclease digestion (M. Jacob). In some cells, the 30S nuclear RNP contains multiple copies of four major structural polypeptides (molecular weight 34,000-40,000) held together by protein-protein interactions (T. E. Martin, University of Chicago), whereas in others as many as 87 different proteins have been identified, including some 20 with molecular weights of 30,000-40,000 (Scherrer). In addition to hnRNA, nuclear RNP particles contain also a low molecular weight RNA (snRNA) which has a longer half-life and appears to be hydrogen-bonded to hnRNA by sequences of some 15-25 nucleotides (A. Alonso, Cancer Centre, Heidelberg).

Free and polysomal messenger ribo-

nucleoproteins present in a wide range of different cells were much discussed. No definite conclusion emerged concerning relationships between any particular protein(s) and the functional state of the messenger, but there is increasing evidence for the view that mRNP proteins may be turning over independently of the mRNA. Thus, proteins associated with free mRNP from muscle cells were reported to be displaced during translation of the messenger in reticulocyte lysates (S. Sarkar, Boston Med. Res. Inst.). The widespread occurrence of RNA-binding proteins in eukaryotic cells (L. P. Ovchinnikov, Molecular Biology, Moscow), as well as the observation that free globin mRNA becomes associated with proteins during translation in a cell-free system from Ehrlich ascites tumour cells (H. R. V. Arnstein, King's College, London), all lend support to the idea of a dynamic state of mRNP proteins despite the well-known strong chemical interaction between the proteins and mRNA.

Differences in mRNP proteins of membrane-bound and free polysomes (N. Standart, Univ. College, Cardiff) and also after virus infection of cells (J. G. Tasseron-de Jong, University of Leiden) suggest possible functions of proteins in relation to the intracellular location of messengers and control of translation. Two other specific proteins, ribophorin I and II, which are present in the rough endoplasmic reticulum of rat liver and other secretory tissues are also involved in binding ribosomes to the membrane (D. A. Sabatini, New York University Medical Center).

Several contributions dealt with the cytoskeleton and its significance for protein synthesis. Thus, polysomal mRNA may be linked to the cytoskeleton by the poly(A) tail and poliovirus mRNA displaces cellular mRNA from intermediate filaments, which may explain the cytopathic effect of poliovirus and other viral infections (S. Penman, Massachusetts Institute of Technology). In lens tissue a particular membrane protein is synthesised by polyribosomes which are specifically bound to the action of the cytoskeletal framework (H. Bloemendal, University of Nijmegen). It was also suggested (A. O. Pogo, N.Y. Blood Center) that in interphase hnRNA is bound to a highly ordered structure formed by non-histone protein interactions and that after post-transcriptional processing mRNA leaves the nucleus already attached to the cytoskeleton. Thus, both processing and transport of mRNA may be dependent on the internal structural organisation of the cell.

In the session on translational control, differences in the distribution of mRNA between the polysomal and non-polysomal cytoplasmic fraction

were reported in Friend erythroleukaemic cells before and after induction of globin synthesis with dimethylsulphoxide (F. Gabrielli, University of Pisa) and in mouse ascites cells (Martin). Also, during the development of the dormant gastrulae of *Artemia salina* changes seem to occur in both the translational efficiency and sequence complexity of mRNA (T. C. James, NIMR, London). Dormant gastrulae contain a unique 19S protein complex, which is an aggregate of 40-50 molecules of a 24,000 molecular weight protein with similar properties to the *Artemia* elongation factor-1_{II} (M. Kondo, University of Antwerp). On resumption of development this complex decreases, suggesting the existence of translational control involving elongation either in addition to or instead of the more usual regulation of initiation. Finally, the apparently endless complexities of translational control were illustrated by the discovery of an 11S cytoplasmic protein which prevents the inhibition of protein synthesis by the haemin-controlled repressor (H. O. Voorma, University of Utrecht). This factor is also able to reactivate reticulocyte lysates after preincubation in the absence of haemin, has no eIF₂ activity and probably functions by restoring the activity of the endogenous initiation factor. □



A hundred years ago

CAPTAIN COOK

It seems on first thoughts rather a strange proceeding to publicly celebrate the centenary of the death of a great man, especially when that death was a murder. But this is what the Paris Geographical Society have arranged to do tomorrow in the case, not of any of their own explorers or navigators, but in the case of England's greatest exploring navigator, Captain James Cook, who was murdered 100 years ago tomorrow by the natives of the Sandwich Islands. Cook, and with him England, owed some gratitude to the French, whose government of the time, though at war with this country, generously gave instructions to their warships and colonial governors, not only not to molest Cook in his pursuit of knowledge, but to render him all reasonable assistance. To him we owe the discovery of the Sandwich, and many other Pacific Islands . . . and proved that [Australia] was unconnected with New Guinea, and above all, dispelled the long-lived illusion of a great southern continent, having been the first to cross the Antarctic Circle. From *Nature* 19, 13 Feb., 334; 1879.

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articles

Thermal-timing instability in neutron stars

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A thermal instability can occur in neutron stars, in which a significant burst of heat is generated in a short period of time. Accompanying this heat pulse is a change in the pulse repetition rate of the pulsar. A perturbation analysis of a simple model shows that the instability becomes easier to trigger as the star's initial temperature is reduced, and that once started it can grow very rapidly. The resulting time evolution of the pulsar angular velocity may resemble a period jump.

It has been known for some time that, if neutron star interiors are superfluid, heat will be frictionally dissipated within them as their rotation slows. It is shown here that in certain circumstances this process can become unstable: that the frictional generation of heat can undergo a runaway, depositing a significant burst of heat in the star in a short period of time.

This runaway is purely thermal in nature: it results from an instability in an energy-generation process. Nevertheless, it has observable effects on the rotation rate of the pulsar. Neutron star interiors rotate more rapidly than their surfaces, and the frictional coupling between the two increases with increasing temperature. A burst of heat therefore tends to cause the star to 'seize' up: the inside slows down, and the outside—the pulsar—speeds up. The thermal instability manifests itself through its pulsar timing effects.

The obvious question is whether such a process has anything to do either with the well-known period jumps that have been observed in the Crab and Vela pulsars, or with the restless behaviour which apparently occurs in all pulsars. This possibility is considered later. However, a less obvious but equally important question, and one that is not considered here is: if the steady-state rotation of a neutron star can become unstable, what is the rotational state of such a star?

The instability in words

Baym *et al.*¹ state that a neutron star consists of a superfluid component and a charged-particle component; the pulsar clock is determined by the rotation of the charges (see ref. 2 for a more detailed review). The two components are coupled together by a frictional force density $\mathbf{F}$:

$$\mathbf{F} = \rho_s \rho_c \mathbf{v} / \rho \tau \quad (1)$$

where ρ_s is the mass density of the superfluid component, ρ_c that of the charged component, $\rho = \rho_s + \rho_c$ is the total density, $\mathbf{v}$ the relative velocity difference between the two components and τ the relaxation time describing interactions between the two. The force ($\mathbf{F}$) slows the rotation of the superfluid. In the steady state the relative velocity difference $\mathbf{v}$ adjusts itself until $\mathbf{F}$ is sufficient to slow the superfluid at the same rate as the charges. This

slowing down, because it is caused by friction, generates heat at a rate $\mathbf{F} \cdot \mathbf{v}$ erg cm⁻³ s⁻¹ throughout the superfluid.

The star also loses thermal energy through radiation (of photons from its surface, for instance). If C is the specific heat of the star as a whole the equation for its temperature T will have the form

$$C \dot{T} = \int \mathbf{F} \cdot \mathbf{v} d^3 r - \{\text{cooling rate}\} \quad (2)$$

Of the quantities entering into the first term on the right-hand side of equation (2) only the relaxation time τ depends on the temperature: τ decreases as T increases (see equation (5)). Therefore, this heating term is an increasing function of T . The same is true of the cooling term. There are only two possibilities: either the heating term increases with temperature faster than the cooling term, or it does not. These two cases are sketched in Fig. 1.

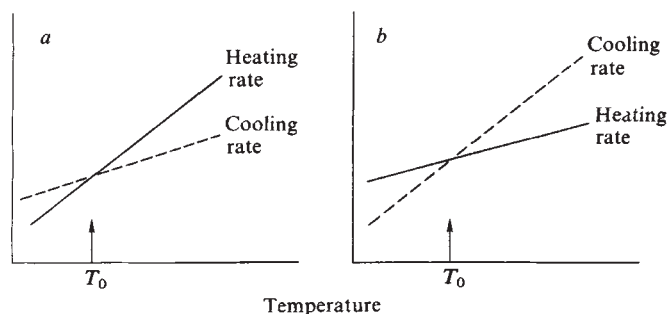


Fig. 1 The dependence on temperature of the heating term (solid lines) and cooling term (dashed lines) of equation (2). The time rate of change of the temperature is determined by their difference. T_0 denotes the steady-state temperature. *a*, Unstable; *b*, stable.

Figure 1 determines the time rate of change of T using the difference between the heating and cooling terms. A time-independent temperature, T_0 , is obtained if they are set equal. This is the steady-state frictionally generated temperature of the star.

Is this equilibrium configuration stable? If we prepare a star with temperature, T_0 , and then perturb the temperature slightly, then Fig. 1 shows that in (a) the perturbation increases in time, while in (b) it dies out. Case (a) is thermally unstable, both against sudden heatings and sudden coolings. Case (b) is stable. A star will be thermally unstable if its energy-generation mechanism is more sensitive to the temperature than is its cooling mechanism.

It is not known whether vortex lines pin to nuclei in neutron star crusts. If they do, a turbulent boundary layer may result, in which the processes described above should take place. However, no quantitative theory of this layer is available.

Perturbation theory on a simple model

Most of the essential features of the instability can be obtained quite easily from the simple two-component model introduced by Baym *et al.*¹ in which the superfluid is supposed to be rotating rigidly. The known pulsars are cold enough for the dominant mode of cooling to be photon emission from the surface. Confining our attention to this case, and combining equations (1) and (2) we obtain after an obvious transformation:

$$C\dot{T} = \frac{I_s I_c}{I\tau} [\omega - \Omega]^2 - 4\pi R^2 \sigma T_e^4 \quad (3)$$

where I_s is the moment of inertia of the superfluid, I_c that of the star, $I = I_s + I_c$, ω is the superfluid angular surface velocity and Ω that of the charges, and T_e is the surface temperature of the star. Here we will take T_e to be proportional to T and neglect the temperature dependence of the specific heat: these assumptions are for convenience only and do not affect the qualitative features of the instability. Equation (3) is then of the form

$$\dot{T} = a/\tau(T) - bT^4 \quad (4)$$

with the factors a and b independent of temperature. (In a perturbation a will change because ω and Ω change. This, however, is a second order effect.)

Consider a different problem: simple cooling from the steady-state temperature with the energy generation mechanism turned off. From equation (4) we find the cooling time $t_{\text{cool}} \equiv (T/\dot{T})_0 = 1/bT_0^3$. This cooling time is of the order of thousands to millions of years for the known pulsars³. The steady-state condition $\dot{T}_0 = 0$ then yields $a/\tau_0 = bT_0^4 = T_0/t_{\text{cool}}$.

The relaxation time τ has been discussed by Feibelman⁴ and Harding *et al.*⁵. Here we adopt Feibelman's result, which can be written

$$\tau = (A/T) \exp\{T_a/T\} \quad (5)$$

In equation (5) A is a complicated function of density only, and T_a is the activation temperature for thermal creation of a vortex core excitation: $T_a = \Delta^2/\epsilon_f k$ where Δ is the superfluid energy gap, ϵ_f the neutron Fermi energy and k the Boltzmann constant.

Now do perturbation theory about the equilibrium temperature T_0 . Writing

$$T(t) = T_0[1 + \epsilon(t)] \quad (6)$$

we obtain from equations (4–6) and the above relationships the perturbation equation for the temperature to first order in ϵ :

$$\dot{\epsilon} = \{[1 + \epsilon] \exp[\epsilon T_a/T_0] - [1 + 4\epsilon]\}/t_{\text{cool}} \quad (7)$$

For infinitesimal perturbations we expand the exponential to find

$$\dot{\epsilon} = \frac{\epsilon}{t_{\text{cool}}} \left[\frac{T_a}{T_0} - 3 \right] \quad (8)$$

In an instability $\dot{\epsilon}$ would have the same sign as ϵ : hot stars are stable, but when the steady-state temperature drops below 1/3rd of the activation temperature the star becomes unstable, both relative to heating and to cooling. The growth time of the instability, however, is of the order of t_{cool} , which is too long to be interesting. Thus the star is essentially stable against infinitesimal perturbations for all temperatures.

The same is true for finite cooling perturbations ($\epsilon < 0$). The case of a finite heating perturbation at low temperatures, however, is quite different. With $0 < \epsilon \ll 1$ but $T_0 \ll T_a$ we obtain

from equation (7)

$$\dot{\epsilon} = \epsilon^{-1} \exp(\epsilon T_a/T_0) \quad (9)$$

in which the exponential can be much greater than unity. Such perturbations are strongly unstable. They grow more rapidly than any exponential with a growth time that becomes far less than t_{cool} .

As a numerical example, the activation temperature in a neutron star is of the order 10^8 – 10^9 K. Suppose we give the star a 10% thermal perturbation. Then the temperature increases with a growth time of the order of the cooling time at 10^7 K, but between 10^4 and 10^{40} times faster at 10^6 K.

As the temperature rises the frictional coupling between the pulsar angular velocity Ω and that of the superfluid ω will increase. The torque equations governing Ω and ω have been written in the two-component model by Baym *et al.*¹ (their equations (1) and (2)). The steady-state solution of these equations is $\dot{\omega}_0 = \dot{\Omega}_0$ and $(\omega - \Omega)_0 = I\tau_0 \dot{\Omega}_0/I_c$. We will look at perturbations in the slowing-down rates $\dot{\Omega}$ and $\dot{\omega}$: write

$$\begin{aligned} \dot{\Omega} &= \dot{\Omega}_0[1 + \delta(t)] \\ \dot{\omega} &= \dot{\omega}_0[1 + \gamma(t)] \end{aligned} \quad (10)$$

Combining equations (6) and (10) with the torque equations we obtain

$$\gamma = -(I_c/I_n)\delta \quad (11)$$

and the basic equation governing the fluctuations in the slowing-down rate of the pulsar (to first order)

$$\dot{\delta} = -\dot{\epsilon} \frac{I_n}{I_c} \left(1 + \frac{T_a}{T_0} \right) - \frac{\delta}{\tau_0} \exp\left\{ \frac{T_a}{T_0} \epsilon \right\} \quad (12)$$

Equation (12) shows that in the absence of thermal perturbations the system is mechanically stable: with $\epsilon = 0$ it reads $\dot{\delta} = -\delta/\tau_0$ which describes the classic spin-down problem of Baym *et al.* On the other hand, the mechanical response to the runaway heating described by equation (9) is, for small δ

$$\dot{\delta} = -\dot{\epsilon} \left[\frac{I_n}{I_c} \left(1 + \frac{T_a}{T_0} \right) \right]. \quad (13)$$

Equation (13) shows that a thermal runaway (positive ϵ) is accompanied by a decrease in the slowing-down rate of the pulsar (negative δ). Because the factor in square brackets in equation (13) is greater than unity, the time scale for this decrease is even shorter than that for the thermal runaway.

Discussion

It is well known that, when born in a supernova explosion, a neutron star will be exceedingly hot: its temperature will greatly exceed the critical temperature $T_a/3$ separating stable from unstable configurations. It will therefore initially be stable against thermal perturbations. What is the subsequent thermal history of the star? I have previously² shown that the steady-state frictionally generated temperature of the star T_0 decreases as the pulsar ages. This means that the pulsar begins life in the configuration shown in Fig. 1*b*. The intersection point between the solid and dashed lines drifts towards lower temperatures as time passes; furthermore, as shown here, while this happens the slopes of these two lines become more nearly equal at their intersection point. When T_0 reaches $T_a/3$ the lines have become parallel at T_0 . The star is now neutrally stable against thermal perturbations. When the temperature drops still further the situation switches to that sketched in Fig. 1*a*, and the star has become unstable.

Because the system is strongly unstable only for finite perturbations we must have some idea as to what could cause such

initial perturbations. The smaller the necessary 'triggering' perturbation, however, the less seriously one need take this requirement. Equation (9) shows that a perturbation to the temperature greater than $\varepsilon_{\min} \approx T_0/T_a \approx T_0/(10^8-10^9)\text{K}$ will grow rapidly. For plausible stellar temperatures this can be quite small. Note also that in the binary X-ray pulsars accreting matter can easily supply the necessary perturbations. Furthermore, in the isolated pulsars a neutron starquake may release enough thermal energy to trigger the instability. As a numerical example, Baym and Pines⁶ estimated that $\approx 10^{41}$ erg can be released in a typical 'small' starquake. At a temperature of 10^6 K this yields a 100% change in the temperature for reasonable specific heats: the growth time for the subsequent runaway is of the order of $\exp\{1,000\}$ shorter than the cooling time.

What is the ultimate fate of the instability? As the temperature increases the pulsar tends to lock rotation with the more rapidly rotating superfluid. (The decrease in slowing-down rate described above is the first stage of this process.) There is no reason to believe that once the perturbation has become large this behaviour suddenly ends. In fact, it will end only when the two components have rigidly locked together. At this point the frictional generation of heat ceases and the star commences to cool. By how much has the pulsar sped up in this process? From considerations of angular momentum conservation it is easy to

show that the fractional change in pulsar period will be

$$\frac{\Delta\Omega}{\Omega} = \frac{I_n}{I} \left(\frac{\omega_0 - \Omega_0}{\Omega_0} \right) \quad (14)$$

that is it is of the order of the initial fractional lag between superfluid and crust. Detailed studies² show that this lag can be quite large: the mechanism seems capable of producing large period jumps such as have been observed in the Vela pulsar. At any rate, the fact that a sufficiently cool pulsar can exhibit the instability demonstrates that its rotational state must be radically different from that of a hotter one.

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Massive deep-sea sulphide ore deposits discovered on the East Pacific Rise

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Massive ore-grade zinc, copper and iron sulphide deposits have been found at the axis of the East Pacific Rise. Although their presence on the deep ocean-floor had been predicted there was no supporting observational evidence. The East Pacific Rise deposits represent a modern analogue of Cyprus-type sulphide ores associated with ophiolitic rocks on land. They contain at least 29% zinc metal and 6% metallic copper. Their discovery will provide a new focus for deep-sea exploration, leading to new assessments of the concentration of metals in the upper layers of the oceanic crust.

THE area of the deposits of ore-grade zinc, copper and iron sulphide was explored and sampled in February–March 1978 by the manned diving saucer CYANA during the expedition CYAMEX¹. The expedition, the only submersible diving programme that has so far been conducted on the East Pacific Rise

(EPR), is part of the French–American–Mexican project RITA (Rivera–Tamayo), a 3-yr study devoted to detailed geological and geophysical investigations of the EPR crest. The ore deposits were sampled in water depths of close to 2,620 m at two neighbouring sites near 20° 54' N 109° 03' W, (refs 2–5) about 90 km north of the Rivera transform fault and 240 km south of the Tamayo transform fault (Fig. 1). Three dives of Cyana (CY 78–06, 08 and 12) crossed the two sampling sites, and we collected samples during two of these dives (CY 78–08 and 12). However, during all dives in the EPR axial zone, signs of hydrothermal activity were seen, including colonies of dead giant clams, fields of pillow lavas with pronounced colour-staining at the base of pillows, and coloured deposits on exposed scarp surfaces of normal faults and open fissures¹. Coral-like growths, possibly of native sulphur, occur in other locations, including a sedimented fault-scarp about 1.0 km to the west of where the sulphide ores were sampled.

Sampling sites

The two sites where the sulphides were sampled lie on the lightly sedimented flanks of steep-sided structural depressions, about 20–30 m deep, 20–30 m wide, and about 600–700 m west of the

*The authors are all members of the CYAMEX Scientific Team.

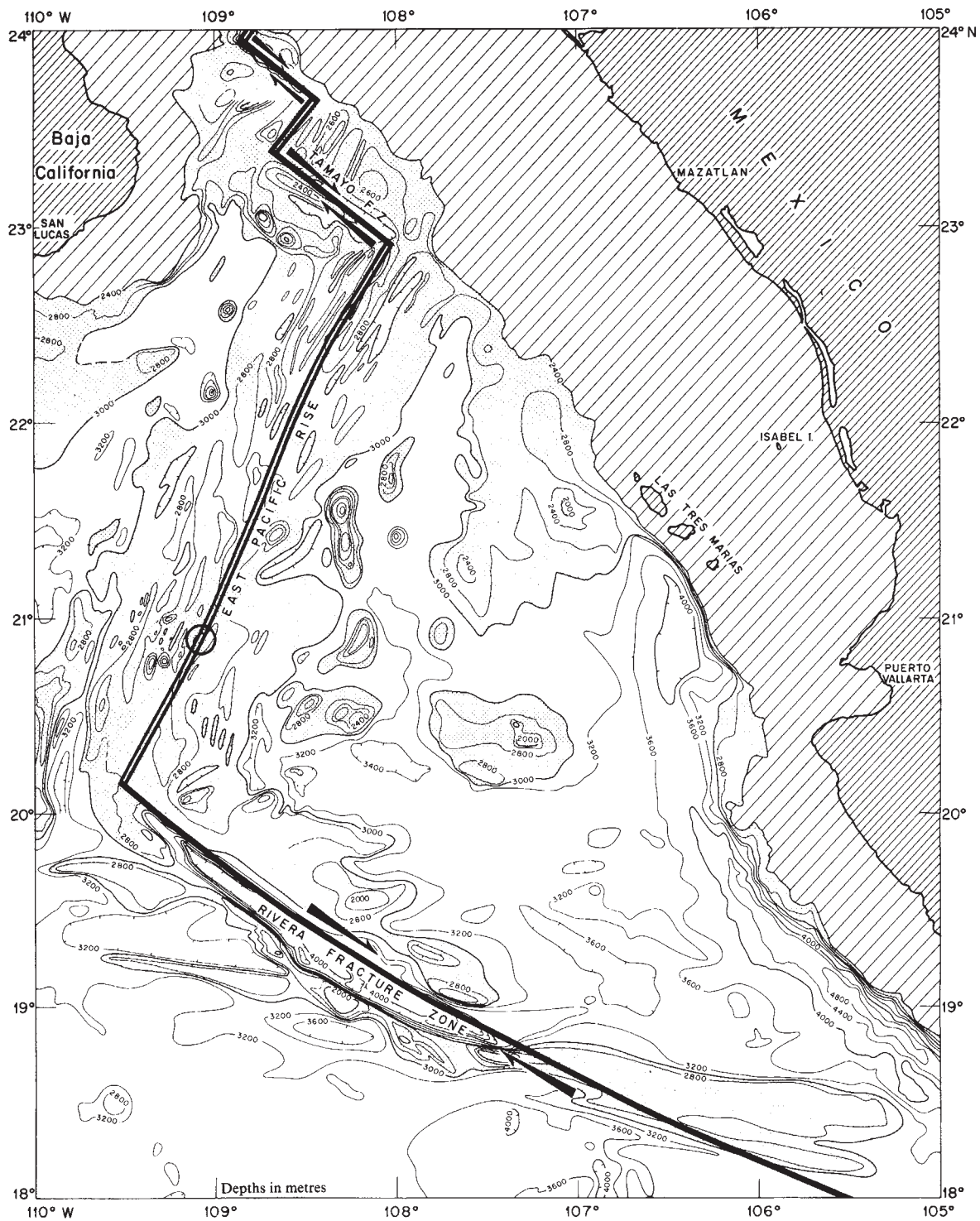


Fig. 1 Setting of the axial zone of the East Pacific Rise where the sulphide ore deposits were explored and sampled (circle).

axis of the 'extrusion zone' where the youngest lavas occur. Whereas the extrusion zone is marked by a 50 m-high sediment-free discontinuous ridge with no fissures or faults, the structural zone within which the sulphide deposits are located is a 1.5 km wide tectonically active band of fissured and/or faulted terrain, the fault pattern being typically of the small horst and graben type and with all the active structures running parallel to the local strike of the EPR.

The massive sulphides were sampled from various parts of tall (up to 10 m-high), extremely irregular columnar edifices about 5 m in diameter (Figs 2 and 3). At one site, explored in relative

details during dive CY 78-08 (Fig. 2), the vertical edifices are 4-5 m apart and are aligned along a direction of 025° over a distance of at least 50 m, in gullies cutting through the 045°-trending, westwards-facing scarp of a structural depression (graben). The second site, explored during dive CY 78-12, is about 200-300 m away and lies on the eastwards-facing flank of a possibly older graben. Here, there is more sediment cover, there are yellow-coloured coral-like growths and the edifices seem to be more indurated. The true spatial relationship of the two sampled sites is not established because of navigational difficulties during dive CY 78-12.

The along-strike extent of the sulphide mineralisation is not known. However, during dive CY 78-08 the graben was followed for about 100 m to the SSW (200°) where it became a 2–3 m wide open fissure with truncated pillows entirely stained by ochre and red-brown deposits exposed on the scarp. The eastern lip of the fissure has small (about 0.5 m high), sponge-like porous edifices and coral-like, bright yellow growths on sediment between pillows. Further south the fissure narrows and is bare of any hydrothermal deposits.

In general, the vertical edifices are built on a pillow lava terrain covered with a light dusting of sediment, the colour of which suggests some contribution of weathering products from the deterioration of the columnar edifices themselves. The edifices are variegated, the dominant colours being ochre, red, yellow, brown, white and black. They are porous and their entire mass is composed of a labyrinth of small channels or tubes separated by thin walls. Their internal structure can be compared to that of a sponge or a mass of clinker or brains. The framework of the edifices is made up of both amorphous, white translucent silica (which has been seen bare in one case) and the sulphides. The presence of open vents on top of the edifices demonstrates that they are constructional features built directly on the ocean crust. For this reason, we described them as hornitos when first observed. The tubes, conduits and channels pervading the mass of the edifices presumably represent the passages through which hot, metal-laden solutions circulated on their exit from the sea floor.

Three other modes of mineralisation occur besides the spectacular vertical edifices: (1) flattish incrustations coating the moderately steep slopes with a rugose, heterogeneous surface texture made up of the same material as the tall edifices; (2) bright yellow or orange, 10–20 cm wide conelets built on sediment and with small holes at their tops and which lie around the large edifices; (3) yellow and red-brown travertine-like flows draping the near-vertical scarps of the graben that contains the row of vertical edifices. No unequivocal bottom-water temperature anomaly was observed during the exploration of any of the mineralised structures, suggesting completed or quiescent hydrothermal activity.

The material collected from each of the two sampling sites was separated on board ship according to colour and shape, giving a

total of 14 samples (three from the site sampled during dive 8 and 11 from that sampled during dive 12). Some of the 14 samples thus designated comprise several subsamples or collections of fragments. A total of 215 subsamples and 11 boxes of small fragments were separated in the laboratory. The samples are very heterogeneous and complex, and no attempt is made at this stage to give a full description of them. We describe here only the gross mineralogy and give an indication of the chemistry of a few important subsamples.

Mineralogy and chemistry

The material consists of friable and porous compounds that are easily fragmented; thus some subsamples are as large as 7 cm in diameter but many are smaller, and some consist of debris with diameters of one to a few mms, or are essentially powder. The subsamples are variegated: yellow to ochre-coloured, grey and blue metallic hues, and grey, bronze red and white reflections. The ochre-coloured material ranges from pale yellowish orange (10 YR 8/6) and moderate yellowish brown (10 YR 5/4) to moderate brown (5 YR 4/4) and from blackish red (5 Y 2/2) to moderate red (5 R 4/6). Most subsamples show well developed cylindrical tubes that display a concentric zonation of medium grey (N 5) layers on the outside to dark gray (N 3) and white (N 4) layers inside. Other tubes are composed of the lighter-coloured moderately brown material (5 YR 4/4). The inner walls of some of the tubes are lined with shiny, silvery deposits that are in part microcrystalline, whereas others are covered only by reddish deposits. Some tubes are lined with black deposits inside and coated with yellow and ochre-coloured deposits outside. Other tubes are filled or partially filled by black material. The tubes are variable in size (0.1–1.5 cm in diameter and up to 8 cm in length) and are irregularly scattered throughout the subsamples. Some tubes are closely spaced in a parallel fashion.

Five pieces from four samples were selected for macroscopic examination and preliminary mineralogical and chemical analysis. Four of the pieces (Cyp 78-12-38A-2, 38A-1-a, 40A-a and 40B) are relatively dense and consist mainly of sulphides. The fifth (Cyp 78-08-14A-1) is oxidised and less dense than the others. Subsample Cyp 78-12-40 B is elliptical in shape; the convex side is coated with dark, heavy sulphide aggregates and

Table 1 Mineralogy and chemistry of hydrothermal samples from 21° N on the East Pacific Rise (major and minor element content determined by X-ray fluorescence and atomic absorption spectrometry)

Subsample or specimen	Approx. size (cm)	Weight (g)	Microscopy		X-ray diffraction		Metal content (wt%)			Other identified elements through XRF
			Minerals	Morphological features	Minerals	Peaks (Å) d(Å)	Fe	Cu	Zn	
CYP 78 12-40 B	6.5×4×4	90.7	Sphalerite	Tabular, polysynthetic twins, octahedron	Sphalerite	3.12; 1.91	9	2.9	23	Co*, Pb*, Ag*, Cd*, Mn*, Cl*, S†, Ca*, K*
			Pyrite	Cubes with striation	Pyrite Chalcopyrite	2.70; 2.42; 1.63 3.05				
CYP 78 12-40 A-a	4×4×1	64.7	Sphalerite	Octahedron, collomorph, banded	Sphalerite	3.13; 1.91	6.3	0.2	28.7	Co*, Pb*, Ag*, Cd*, Mn*, Cl*, K*, Si†, S†
			Amorphous silica	Flaky	Pyrite Chalcopyrite (?)	2.71; 2.42; 1.63 3.06 (?)				
CYP 78 12-38 A-2	4×4×2	26.5	Pyrite or chalcopyrite or sphalerite	Globular and collomorph, tetrahedron	Pyrite Chalcopyrite	3.13; 2.71; 1.63 3.06	29.6†	6†	non analysed	Co*, Cd*, Pb*, Mn*, Zn*, Ca*, K*, Cl*, S†
CYP 78 12-38 A-1-a	4×2×2	74.3	Pyrite	Cubes in lamellae, octahedron with striations	Pyrite	2.71; 2.42; 1.63	14.9	2.2	0.0	Mn*, Cd*, Co*, Pb*, Ag*, Zn*, K*, S†
			Pyrite or chalcopyrite or sphalerite	Globular and collomorph	Marcasite Chalcopyrite (?)	3.44; 2.71; 1.76 (3.04)?				
CYP 78 08-14 A-1	6×5×1.5	19	Iron oxides	Globular aggregates	Amorphous	Broad	42.7§	0.0	0.6	Mn*, Pb*, Cu*, Ca†, Cd*, K*, S†

*Few tens to few thousands p.p.m. †Few % to few tens of %. ‡ Analysed by XRF. § Red part of subsample CYP 78-08-14A-1.

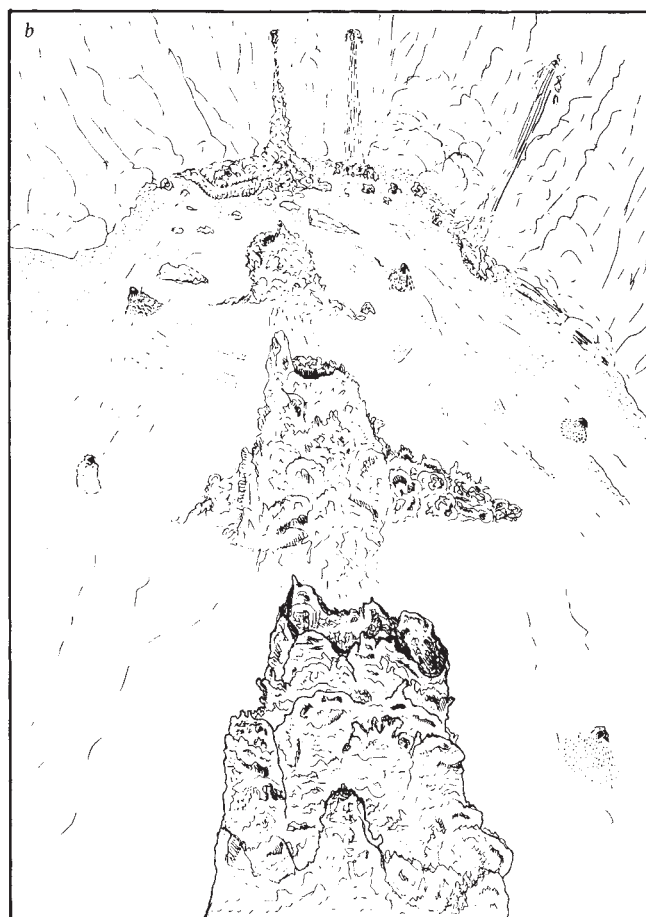
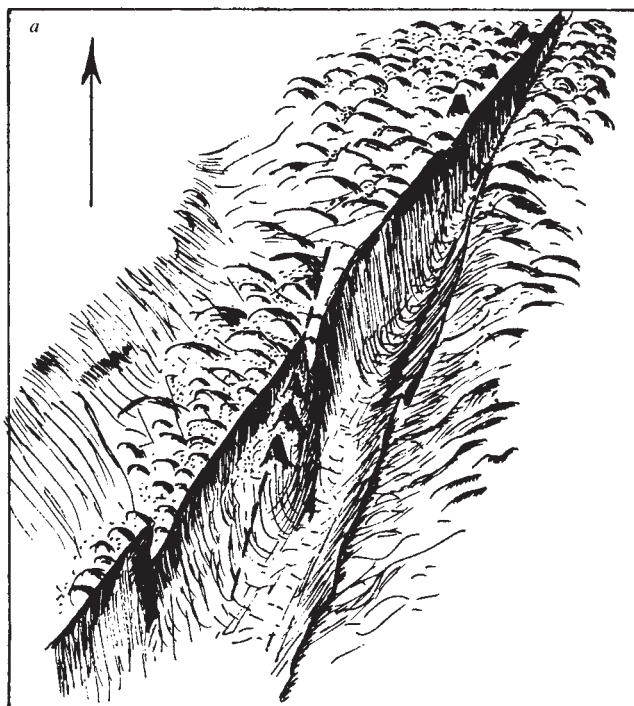


Fig. 2 *a*, Overall sketch of the 225° trending structural depression with a small gully or re-entrant trending 205° that crosses the east wall of the graben (dive CY 78-08). The width of the graben is about 20–30 m and narrows towards the SSW. The arrow points towards the south. *b*, Artist's view from CYANA's porthole of the site of sulphide ore edifices on the eastern flank of a structural depression (dive CY 78-08). The view is towards the south. The hornito-like edifices are aligned along 205 in a gully cutting across the graben. The spacing between 'hornitoes' is about 5 m. The small conelets associated with the vertical edifices are shown as well as the flows of hydrothermal material on the vertical scarp in the cul-de-sac of the small gully. All the sulphide material was sampled from the porous edifices.

there are patches of greenish-yellow dust with a strong odour of sulphur. The concave surface of the specimen is coated by moderate yellowish brown and brownish ochre material. Small empty tubes (0.1–1 cm in diameter) are also seen. Specimen Cyp 78-12-40A-a is a nearly black material with circular tubes, one of which has its internal wall coated by a brass-yellow lamella. Concentric zonation of dark (outside) and white (inside) lamellae is exhibited by another adjacent tube. Specimen Cyp 78-12-38A-2 is dark grey with remnants of tubes and patches of yellowish-green, green and purple brown material similar to the other dark sulphide aggregates. Specimen Cyp 78-12-38A-1-a is a massive aggregate of black sulphide-rich material which is extremely friable and porous and gives off a strong odour of sulphur. It contains specks of yellowish-green material which could represent a sulphur oxide compound. Subsample Cyp 78-08-14A-1 is a moderate brown to blackish red to ochre-coloured powdery material with abundant relicts of tubes, some of which are open and continuous across the specimen, while others are filled by a yellowish brown material. Sulphide material coats some of the walls of the empty tubes.

The mineralogy of the material was determined by X-ray diffraction after microscopic examination in reflected and transmitted light (Table 1). The ochre-coloured material consists mainly of amorphous iron-oxide. The oxides are reddish and yellow and are found in the interstices of the sulphide grains, resulting primarily from secondary alteration of the sulphides.

The sulphide phases consist mainly of sphalerite and pyrite with minor chalcopyrite and marcasite. The sulphides are represented by agglomerates of intimately associated, well-

crystallised phases and amorphous material. The most common form encountered in the samples is an octahedron. Tetrahedra characteristic of sphalerite, cubes of pyrite, bipyramids, sphenoids characteristic of chalcopyrite, and tabular crystals with polysynthetic twins (probably of sphalerite) are also observed. Globular and reniform associations, probably consisting of chalcopyrite and pyrite, with anisotropic clay-size material are commonly associated with the well-crystallised sulphides.

The chemistry of the five specimens was determined qualitatively by X-ray fluorescence (XRF) spectrometry (dispersive) and quantitatively by atomic absorption spectrometry (Table 1). The XRF analyses show the presence of abundant zinc, iron, copper and sulphur and of lesser quantities of several other elements including cobalt, lead, silver and cadmium. The material used for atomic absorption analyses (100 mg to 1 g) should be reasonably representative of the subsamples which weigh from 19 to 91 g. The analyses show two principal modes: one zinc-rich (23–28.7%) and the other iron-rich (19.9–42.7%). Three specimens are copper-rich (2.2–6%) and we note the absence of manganese even as a minor element.

The subsamples can be divided into two major types according to their macroscopic features, mineralogy and chemistry: (1) the dark-coloured and porous, zinc, iron and copper sulphides in the form of sphalerite + marcasite, pyrite and chalcopyrite, with a skeleton made up of a white unidentified material; and (2) the fragile, pulverulent ochre-coloured material made up of amorphous iron oxides. The widespread occurrence of pure sulphur on many of the subsamples suggests that the small yellow conelets and bright yellow crystal-like growths around them,

which are associated with the larger variegated edifices on the sea floor, might represent massive concentrations of native sulphur.

The occurrence of sulphide mineralisations on the sea floor in an active tectonic region directly associated with an accreting plate margin can best be explained by a model in which the deposits formed within the discharge zone of a hydrothermal circulation system involving seawater as the convecting fluid⁶⁻¹². A similar explanation has been put forward for the genesis of ophiolitic sulphide ore deposits on land, which share many common characteristics with the EPR deposits^{6,9-17}. A typical example of the ophiolitic sulphide ores is found in Cyprus^{6,10,12,13-15,17-20}.

The Cyprus deposits occupy basin-like depressions in basalts and occur as irregular, elongate lenticles or lens-shaped bodies of massive sulphide ores (pyrite + chalcopyrite ± sphalerite ± marcasite with minor galena, pyrrhotite, gold and silver). Like the EPR deposits, which are also restricted to structural lows, they accumulated on the sea floor before the first sediments were deposited and in some deposits the ore is overlain by pillow lavas and sediments. An interval of 10^5 years has been tentatively proposed for the formation of the Cyprus ores¹². The EPR deposits are adjacent to the main axial extrusion zone but there is direct evidence from the dives that lava can also be emplaced within the horst and graben tectonic zone so that burial of the deposits by off-axis volcanism is possible. We estimate the age of the EPR deposits to be $<1-2 \times 10^4$ years using as a guide the fact that they occur on crust whose age is theoretically $1-2 \times 10^4$ years, based on spreading rate.

The Cyprus deposits can show alteration near the top to a geothitic material or to pyrite-bearing chemical sediments (ochres) enriched in silica and hydrated iron-oxides. The ochreous components of the EPR deposits are probably gossans and represent products of submarine weathering of the sulphide ores. The Cyprus deposits display prominent metal zoning with zinc-rich bodies lying deep in the Lower Pillow Lavas and larger copper-rich bodies at shallower levels in, or at the top of, the Upper Pillow Lavas. We have no evidence yet of whether or not there is metal zoning within the metre-scale sulphide cones of the East Pacific Rise. There are indications of some zoning at the scale of the samples. The sulphides on Cyprus exhibit primary breccia textures in the upper part and are massive in the lower part. Ore textures similar to the sponge-like texture of the EPR deposits have not been described in Cyprus or other Cyprus-type sites but the Cyprus ores are said to be very porous. The fragile tubular and heterogeneous texture of the EPR sulphides may not be preserved in the geological record or possibly they represent the equivalent of the upper breccias in Cyprus ores. Finally, the Cyprus ore deposits are separated by a mean distance of about 2.5 km in a direction perpendicular to the strike of the sheeted dikes and thus to the strike of the fossil spreading centre. The hydrothermal sulphur/sulphide sites of the EPR are separated by about 1 km in a direction transverse to the EPR axis.

Conclusions

Despite the differences in detail and in scale between the East Pacific Rise deposits and the Cyprus ores, there are enough general similarities in the type and style of metal concentration to regard the EPR deposits as a modern analogue of the zinc-rich cupriferous massive sulphide deposits associated with ophiolite complexes. The EPR ore deposits, which have their primary features still preserved and which have been emplaced in a well defined geological setting directly associated with plate accretion, should shed a new light on the characteristics of, and genetic models for ophiolitic sulphide ore deposits such as found in Cyprus^{6,10,12,13-15,17-20}, Newfoundland¹⁶, California¹⁰, Turkey¹⁰, and Italy⁹. The EPR deposits further demonstrate that modern ocean floor sulphide deposits are not restricted to the initial stages of ocean rifting, as suggested by the Red Sea deposits.



Fig. 3 Photograph of a variegated hornito-like edifice taken during dive CY 78-06. The diameter of the edifice is ~2 m. A second edifice can be seen in the background.

The metal-rich brines and muds that are found in the axial region of the Red Sea²¹, or beneath the Salton Sea Trough²² (a northwards extension of the Gulf of California) show iron-rich layers overlying a sulphide horizon with sphalerite and less abundant chalcopyrite, pyrite and marcasite. In contrast with the sulphide-rich, Mn-deficient EPR deposits, the other metal-liferous deposits encountered in Mid-Ocean Ridge settings are essentially Fe-Mn concretions, Fe-Si rich clay material, and occasional pure MnO₂ deposits²³⁻²⁶.

Mid-Ocean Ridge sulphides and ferromanganese deposits attributed to a hydrothermal origin are all chemical precipitates which form at the basalt-seawater interface when hot seawater solution, laden with Fe, Mn and other metals stripped at 'high temperature' (see refs 8, 10, 12) from the ocean crust, debouches onto the ocean floor. Sulphides, such as the EPR deposits, can be preserved either if the bottom waters are anoxic or if the sulphide deposits become rapidly buried under sediments or lavas. The ochres, such as those of the East Pacific Rise, result from the oxidation of the iron sulphide. The Fe-Mn deposits (umbers) encountered in the FAMOUS area, in the Galapagos and in the Gulf of Aden may be the product of a weaker flow of the convecting fluid although many other factors (such as temperature, redox conditions, and fugacity) may be critical. Sulphides in such settings would be deposited sub-surface. Indeed, this is suggested in the FAMOUS area by the presence of pyrite in the deposits near the vents²⁴. It is not clear whether the flow rate of ocean floor convective systems depends primarily on the spreading rate but it seems from the contrast between the RITA and Galapagos areas and the FAMOUS area that hydrothermal activity is more prevalent in higher spreading-rate environments.

It will be a test of ingenuity to assess the surface distribution of metal-rich deposits on the Mid-Ocean Ridge, let alone their distribution at depth. Furthermore, there is the problem that unusual circumstances might be required for their preservation. However, the discovery in the RITA area shows that metal deposition is not restricted to young oceans and that the axial zones of medium-fast (and perhaps fast) ridges are not only factories of oceanic crust and lithosphere but also plants for the concentration of heavy metals through leaching of the oceanic crust in zones of intense hydrothermal activity and deposition on the basaltic floor. Indeed, the EPR deposits throw a fresh light on the whole question of the concentration of metal deposits of the oceanic crust.

In the future one might envision that submarine metalliferous ore deposits are distributed in quasi-continuous, linear strips several thousand kilometres in length along the axis of the

fast-spreading East Pacific Rise and south-east branch of the Mid-Indian Ocean Ridge. Certainly we will have to re-examine our estimates of the mineral resources which lie within reach on top of the bare crust of the Mid-Ocean Ridge. Slow ridges seem so far to yield only Fe and Mn-rich deposits in fracture-zone terrain; thus, the sites of priority interest for further sulphide ore exploration are the fast as well as super-fast ridges.

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Friction and seismic attenuation in rocks

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Precise experimental results, combined with theoretical predictions, indicate that seismic energy loss caused by grain boundary friction is important only at low confining pressure and at strains greater than about 10^{-6} . Since these conditions are generally not encountered in seismology, frictional attenuation is not important in situ. Other mechanisms such as fluid flow must dominate seismic attenuation in the upper crust.

FAR more information about the constitution of the Earth is obtained from seismic studies than from all other geophysical methods combined. Of the two main aspects of wave propagation—velocity and attenuation—knowledge of velocities has provided most of our information about the Earth. Attenuation data is of much more limited use, partly because it is difficult to obtain, but mainly because it is difficult to interpret in terms of rock properties. This is due primarily to our lack of understanding of the physical processes involved in the attenuation of seismic waves. (Here we are not considering apparent attenuation caused by geometrical spreading, partial reflections and so on, but are only interested in those processes that convert seismic energy into heat.)

Many different techniques have been used to study the attenuation of acoustic waves propagating through rock. Although much data has been collected, no well-defined mechanism of energy loss has yet been firmly established on both experimental and theoretical grounds. One of the most intuitively appealing and widely discussed mechanisms proposed for seismic energy loss is based on simple Coulomb friction. In this mechanism the passing wave causes sliding at grain boundaries or across crack faces, thereby doing work against friction and converting seismic energy into heat. Since frictional heating is rate independent, it seems to account for the

reported observation that the specific dissipation function¹, Q , is nearly independent of frequency. Friction also seems to explain why the introduction of cracks into a crystalline solid increases attenuation. The most severe criticism of a frictional mechanism is, however, that the mechanism is inherently nonlinear. This means that the velocity and attenuation of a sinusoidal wave will depend on how it is combined with other waves. It has been found that at large strain amplitudes ($>10^{-6}$) nonlinear effects such as cusped hysteresis loops^{3,4} and amplitude-dependent velocity and attenuation^{3,5,6} can be observed, but these effects disappear at strains more typical of seismic waves.

Using both theoretical arguments and experimental results, several authors^{2,7–9} have expressed doubts about the validity of a frictional attenuation mechanism. For instance, Savage⁷ has suggested that frictional effects should be important only at large strains where displacements across crack faces are sufficiently large for the concept of macroscopic friction to be valid. Despite these criticisms, frictional losses are often used to interpret experimental observations^{10–12}. To clarify the role of friction in seismic wave attenuation, we carefully investigated the dependence of velocity and Q on strain amplitude. We were able to identify the range of conditions for which frictional losses may be significant and our results show that the frictional mechanism is generally not important for seismic waves in the Earth.

Theory of frictional losses

Two types of frictional attenuation models have been proposed. Walsh¹³ developed a model based on thin elliptical cracks in which frictional sliding occurs between crack faces. Models based on spheres in contact were developed by Mindlin and Deresiewicz¹⁴ and by White¹⁵. Mavko¹⁶ has reviewed these theories and extended the contact model to more general geometries. The most significant feature of Mavko's model (which it shares with that of Mindlin and Deresiewicz) is that it predicts Q^{-1} to be proportional to strain amplitude. It has been

found experimentally that frictional behaviour is often described by an equation of the form¹⁷

$$\sigma_t = S + \gamma \sigma_n$$

where σ_t is the frictional force, S is the cohesive shear strength, γ is the coefficient of friction and σ_n is the normal stress across the sliding surfaces. The contact models predict that attenuation will decrease as either γ or S increases. Attenuation will also decrease as σ_n increases and the sliding interfaces are effectively locked together.

In addition to the effects on attenuation, all frictional models predict that stress-strain loops will have discontinuities in slope at maximum strain. This is a static hysteresis effect. As stress reverses direction there is a simultaneous reversal of elastic strain and a delayed reversal of sliding strain.

Table 1 Sample descriptions

Sample	Dimensions (cm)	V_E/f_E (km s ⁻¹ Hz ⁻¹)	Porosity
Massilon sandstone	100 × 2.0 × 1.9	2.000	22%
Berea sandstone	101.6 × 2.54 × 2.54	2.032	20%
Sierra White granite	101.6 × 2.54 × 2.54	2.032	1%
Vycor 7930 glass	91.4 × 1.5 diam.	1.828	28%
Lucite (plastic)	98.7 × 1.51 diam.	1.974	0%
Aluminium	100 × 2.22 × 2.22	2.000	0%

Vycor 7930 porous glass is made by Corning Glass Works; the average pore size is 4 nm.

Past observations of frictional losses

Several of the predicted effects of frictional attenuation models have been observed. Energy loss has been found to increase with strain amplitude at large strains in rock³ as well as between sliding glass and metal surfaces^{19,20}. It has been shown that at low strains (<10⁻⁶) attenuation is nearly constant³ and that variation with strain amplitude occurs only at larger strain. Some attenuation in metals at large strain is associated with dislocation mechanisms, but this does not explain all of the observations.

Discontinuities in the slope of stress-strain loops have been observed in large strain quasi-static experiments^{3,4}. These have been described as cusped loops. It has recently been shown²¹ that as strain amplitude decreases, the cusped loops become elliptical at strains approaching 10⁻⁶. It is interesting that two of the effects predicted by frictional attenuation models, amplitude-dependent Q and cusped hysteresis loops, both seem to disappear below the same strain amplitude.

It is also commonly observed that increasing confining pressure causes attenuation to decrease^{22,23}. This is predicted by frictional models, but it is also predicted by many other possible mechanisms of energy loss and so by itself does not provide evidence for frictional loss.

New observations of frictional losses

We are using the well established bar resonance technique^{22,23} to measure both attenuation and velocity in long thin bars of rock. (Experimental details will be presented elsewhere.) An important feature of our experiment is that pore pressure is controlled independently of confining pressure. This has not previously been achieved in a resonance experiment. The length of the samples (~1 m) puts the fundamental resonance frequencies at roughly 500–1,500 Hz. Sample dimensions are given in Table 1. Resonance frequencies are measured to 1 part in 10³ and converted to velocities using the relation $v = 2Lf$ where L is the length of the bar. Geometric corrections given by Spinner and Tefft²⁴ are negligible for our samples in extensional resonance. Strain amplitudes are measured with crystal phonograph pick-ups calibrated against semiconductor strain gauges.

Table 2 Tests for nonlinear behaviour in various materials (negligible changes of Q_E and V_E are found for materials where frictional mechanisms are inoperative)

Material	Strain amplitude	Q_E	Velocity (m s ⁻¹)
Lucite	1.43 × 10 ⁻⁶	23.4 ± 0.3	2,108
	3.04 × 10 ⁻⁸	23.2 ± 0.4	2,108
Vycor	2.84 × 10 ⁻⁶	437 ± 2	3,345
	1.58 × 10 ⁻⁸	435 ± 4	3,345
Aluminium	2.41 × 10 ⁻⁶	34,800 ± 300	5,102
	2.46 × 10 ⁻⁸	35,200 ± 400	5,102
Granite	1.44 × 10 ⁻⁶	185 ± 1	3,629
	4.15 × 10 ⁻⁸	204 ± 1	3,637
Massilon sandstone	2.10 × 10 ⁻⁶	107 ± 1	1,820
	1.55 × 10 ⁻⁸	138 ± 2	1,834
Berea sandstone	2.10 × 10 ⁻⁶	103 ± 3	1,937
	2.30 × 10 ⁻⁸	140 ± 2	1,955

Attenuation measurements can be made either by plotting resonance peaks ($Q = f/\Delta f$) or by measuring the time constant of resonance decays ($\tau = Q/\pi f$). Before using either technique, resonance peak shapes were carefully observed to insure the purity of the resonances. For convenience, all attenuation data presented in this article was taken with the resonance decay technique (except as noted in Fig. 1). The precision of the attenuation measurements is 1% and the estimated absolute accuracy is 5–10%. Both torsional (shear modulus) and extensional (Young's modulus) resonance modes show similar nonlinear behaviour at large strains. Only extensional data (taken at the fundamental resonance) will be presented here.

Data for dry Massilon sandstone as a function of resonant strain amplitude are shown in Fig. 1. At strains below 5×10^{-7} both attenuation (Q^{-1}) and velocity are nearly independent of strain amplitude, whereas at relatively large strains (>10⁻⁶) there is a clear increase in attenuation and decrease in velocity. Of the two measured quantities, Q^{-1} is more sensitive to strain

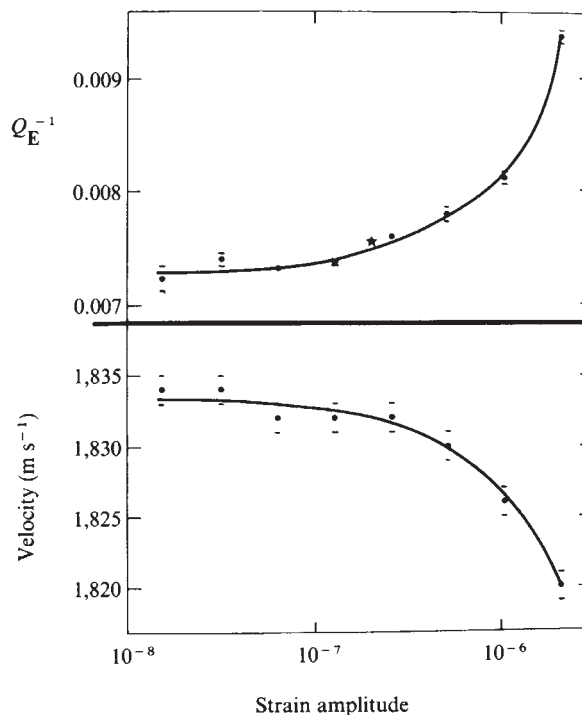


Fig. 1 Variation of attenuation (Q^{-1}) and velocity with strain amplitude for dry Massilon sandstone in extensional resonance. ★, The value found from the resonance peak half-width using the relation $Q^{-1} = \Delta f/f$.

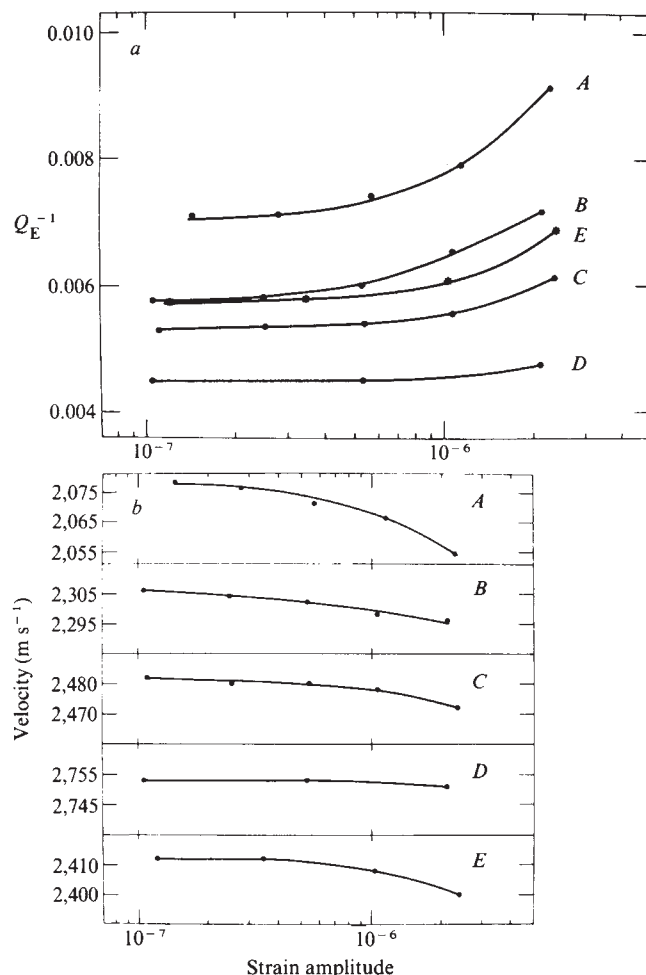


Fig. 2 *a*, Attenuation in dry Berea sandstone as a function of strain amplitude, confining pressure (P_c) and pore pressure (P_p). Helium was used as the pressure medium. (A) $P_c = 10$ bar; (B) $P_c = 20$ bar; (C) $P_c = 30$ bar; (D) $P_c = 50$ bar; (E) $P_c = 50$ bar, $P_p = 30$ bar. *b*, Extensional velocity of Berea sandstone. Data correspond to the attenuation data shown in (*a*).

amplitude than is velocity, varying by 19% and 0.7%, respectively. The similarity of the curve shapes suggests an underlying relationship between the amplitude dependence of Q^{-1} and velocity.

We have further investigated these nonlinear effects by varying effective stress—the difference between confining pressure and pore pressure. Figure 2 shows data for a jacketed sample of Berea sandstone, with helium used for both confining pressure and pore pressure. As confining pressure is increased from 10 to 50 bar (curves A–D), the variation of both Q^{-1} and velocity with strain amplitude gradually decreases. At a constant confining pressure of 50 bar (curves D and E) increasing pore pressure restores the dependence on strain amplitude. In Fig. 2*a* curves B and E show that Q^{-1} is almost a unique function of effective stress at a given strain amplitude.

The effects of changing water content on Q^{-1} in Berea sandstone are shown in Fig. 3. The data for partially saturated rock at very low saturation were obtained at ambient room conditions. Data for 'dry' rock were obtained after drying the sample in a vacuum oven, and the data for saturated rock after saturating the sample with distilled water. The most noticeable feature is that the addition of water greatly increases the attenuation. This effect, which is associated with a fluid flow mechanism, will be discussed more thoroughly elsewhere²⁹. Of greater interest here is that the addition of a small amount of water greatly increases the variation of Q^{-1} with strain amplitude, whereas continued addition of water causes a smaller

additional variation in Q^{-1} . This is indicated by the brackets in Fig. 3 which show the difference between Q^{-1} at a strain of 1.7×10^{-6} and the constant value of Q^{-1} at low strains.

To find out which structural features of a material are related to these nonlinear effects, we experimented with materials having physical compositions different from sandstone. Table 2 shows data for various materials measured at both large and small strain amplitudes. Lucite and aluminium show no evidence of any change in velocity or attenuation with strain (to within experimental uncertainty) although their respective Q values span more than three orders of magnitude and their internal structures are quite different. (The values of Q for aluminium are probably underestimates because they are at the limits of sensitivity of our system.) There is also no variation in the values for dry Vycor glass. Under the scanning electron microscope Vycor appears to be composed mainly of glass beads ~ 50 nm diameter. The contacts were beyond the resolution of the SEM used, (86,000 $\times$), but presumably the particles are welded together since they formed simultaneously from the melt. The flat cracks and partially cemented grain boundaries commonly found in rocks are probably scarce in Vycor. Table 2 also includes data for a room dry granite sample which shows the same nonlinear effects found in sandstone.

Discussion

The data we have presented, together with the existing data we have discussed, provide strong support for the existence of a frictional attenuation mechanism in rock at large strain amplitudes and low effective stresses. Every predicted feature of the

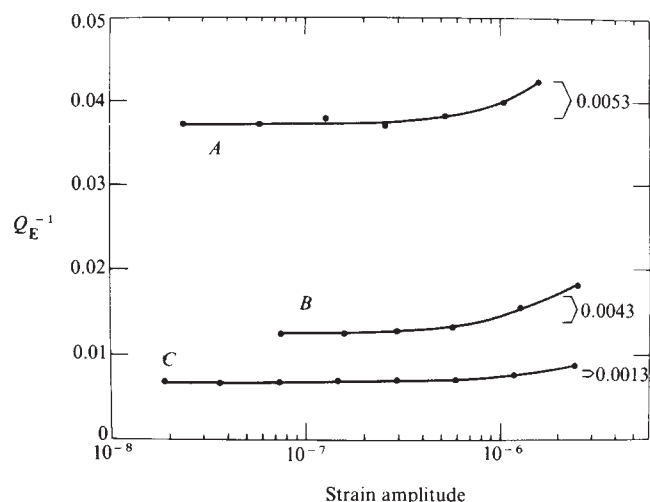


Fig. 3 Attenuation in Berea sandstone at various degrees of saturation as a function of strain amplitude. Brackets indicate frictional component of energy loss. A, saturated; B, partially saturated; C, dry.

contact frictional loss mechanism has been observed experimentally. At large strains ($> 10^{-6}$) Q^{-1} increases with increasing strain amplitude and the amount of the variation in Q^{-1} is strongly influenced by effective stress. A stress of 50 bar is enough to virtually eliminate the strain dependence at the strains and in the rocks which we have used.

The effect of water on the amplitude dependence of Q^{-1} is probably caused by water changing the frictional properties of the sliding interfaces. Figure 3 provides evidence for this. Adding a small amount of water to the sample presumably wets most of the internal surfaces, causing a slight increase in the low strain, linear attenuation (a fluid flow mechanism) and a large increase in the nonlinear, frictional component of attenuation. The addition of more water fills the remaining pore space, thus enhancing the flow losses but only slightly increasing the frictional energy loss.

The observations of cusped stress-strain loops^{3,4} at strains greater than 10^{-6} also indicate that frictional sliding is occurring. It is significant that all of the evidence for a frictional attenuation mechanism disappears below strains of the order of 10^{-6} . The cusped loops become elliptical and Q^{-1} becomes independent of strain amplitude. The implication is that at low strains attenuation is caused only by linear loss mechanisms. At larger strains, nonlinear frictional losses become observable. This leads to the question of why a strain of 10^{-6} or larger is needed before frictional attenuation is observed. We can assume that displacements across crack surfaces must be at least comparable with inter-atomic spacings ($\sim 10^{-8}$ cm) for friction to be present. A shear strain ϵ will produce a displacement d across the sides of a crack of length L given approximately by $d = \epsilon L$. If $d = 10^{-8}$ cm and $\epsilon = 10^{-6}$, then we find that $L = 10^{-2}$ cm. A crack length of 10^{-2} cm may well be a rough upper bound to the majority of crack lengths found in rock. Hadley²⁵ reported that in Westerly granite only 7% of the observed cracks had lengths greater than 10^{-2} cm and none were longer than 3×10^{-2} cm. Therefore, at strains below 10^{-6} , sliding displacements between crack faces are so small in the majority of cracks that friction does not adequately describe the interaction. As strain increases above 10^{-6} , frictional sliding begins to occur at more and more cracks and attenuation increases.

After eliminating friction as a loss mechanism at low strains, we still have to account for the observed energy loss at low strain amplitudes in 'dry' rock. We must also explain the change in attenuation that occurs as cracks are closed under confining pressure^{3,22,23} or created through thermal cracking³ or dilatancy¹¹. Unfortunately, we do not know yet what mechanism is responsible for these losses. The data collected by Tittmann *et al.*⁹ showing the strong effect that trace amounts of volatiles have on attenuation probably has a direct bearing on this mechanism. Another possibility is some form of grain boundary relaxation^{26,27} or a dislocation mechanism similar to those discussed by Mason^{8,28}. Whatever the cause may be, it is reasonably certain that the mechanism does not involve any macroscopic concept of friction.

Conclusion

On both experimental and theoretical grounds it seems clear that frictional energy losses occur in rock at room pressure only at strains greater than approximately 10^{-6} . This is far greater than strain amplitudes generally encountered in seismology

except in regions near seismic sources. Even there the effect of confining pressure will virtually eliminate frictional energy losses in the Earth. Consequently, frictional losses are not important in seismology.

The mechanism by which cracks influence attenuation at low strains in 'dry' rock needs to be re-examined. The concept of frictional energy loss is so firmly established (on intuitive grounds) that the term 'internal friction' is often interpreted literally to mean a frictional energy loss. Though the choice of words is unfortunate, internal friction is simply a general term for energy absorption and does not imply any particular mechanism. It must be kept in mind that actual frictional energy loss is a large-strain, low-pressure phenomenon that is often observed in the laboratory, but seldom observed in the Earth.

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Radiation-induced chromosome aberrations in nuclear-dockyard workers

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The incidence of chromosome aberrations in peripheral blood lymphocytes of 197 dockyard workers has been followed over a 10-yr period. These workers were exposed to mixed neutron-γ radiation during the refuelling of nuclear reactors, but most exposures were below the internationally accepted maximum permissible level of 5 rem per yr. There was a significant increase in chromosome damage with increasing exposure; aberration frequency was a linear function of dose and was influenced by age and time of blood sampling after exposure.

CYTOGENETIC studies on human peripheral blood lymphocytes exposed to low doses of ionising radiation *in vitro* have shown that it is possible to detect significant levels of

chromosome damage from the analysis of a few hundred cells. For example¹, the frequency of dicentric and ring aberrations in nonirradiated control blood cells was found to be 1 in 10^3 and this increased approximately 20-fold after *in vitro* exposure to 30 rad of X rays, the incidence increasing linearly over the range 0-50 rad with no evidence for a threshold dose level. At higher exposure levels, the contribution of a 'dose-squared' component becomes more significant, so that over higher ranges of exposure the yields of such aberrations may approximate to the square of dose. In contrast, the aberration frequency after *in vitro* exposure of blood cells to high linear energy transfer radiations, such as 0.7-0.9 MeV fission neutrons²⁻⁴, increases linearly over a wide dose range and such radiations are considerably more efficient than X rays, per unit of absorbed dose, at dose levels over a range (below a few hundred rad) yielding less than an average of one dicentric aberration per cell.

Studies on chromosomes of lymphocytes from people exposed to ionising radiation have also revealed increased aberration yields after *in vivo* exposure to relatively low doses of X rays, γ rays or fast neutrons. Moreover, because of the equivalent sensitivity of lymphocyte chromosomes to radiation damage *in vitro* and *in vivo*⁵, and because of the strict relationship between aberration frequency and radiation dose and quality *in vitro*, it has become standard practice to use lymphocyte aberration frequencies as a 'biological dosimeter' in cases of accidental *in vivo* exposure to radiation^{6,7}. Extrapolation from *in vitro* studies suggests that an acute X-ray exposure of 5 rad (equal to the maximum permitted annual dose for a radiation worker) would increase the spontaneous frequency of dicentric aberrations by a factor of three or four, so that the 'doubling dose' for these somatic genetic effects is quite small. However, there is little direct information on the possible effects of low dose *in vivo* exposures. A study of workers employed at a UK Atomic Energy Establishment showed that those occupationally exposed to mixed neutron- γ radiation, at levels below the maximum permissible exposure levels, had a significant excess of blood lymphocytes containing dicentric or ring aberrations relative to on-site controls⁸. However, no quantitative dose-effect relationship was evident, possibly because of inadequacy of the control data, a varying pattern of dose accumulation with time between individuals receiving similar total doses, too small a number of subjects or too few cells scored.

The population and methods of analysis

In 1968 the opening locally of an establishment for servicing and refuelling nuclear powered submarines provided an opportunity for studying a population of men subject to occupational radiation exposure within the maximum permissible limits of 5 rem per yr. As the majority of the workers at the new establishment had not received previous occupational exposure to radiation, blood samples were taken before their classification as 'radiation workers', to give background control samples, and serial blood samples were later obtained as dose was accumulated. This population has now been studied over a 10-yr period and chromosome aberrations scored in blood lymphocytes of 197 men, the majority of whom have been sampled on several occasions.

A heparinised venous blood sample of 5 ml was taken at the time of a workers 'preclassification' examination, or annual medical check up, and set up in culture later on the same day. Blood culture and chromosome preparation techniques were those that have been standard in this laboratory⁹ throughout the study period, with all cultures incubated for 48 h at 37 °C. Preparations were stained with orcein and for each culture 100 cells with 45 or more centromeres were scored directly under the microscope by one observer for dicentrics, rings, acentric fragments, minutes, abnormal monocentrics, additional or absent monocentrics (aneuploidy), a medium-sized chromosome with

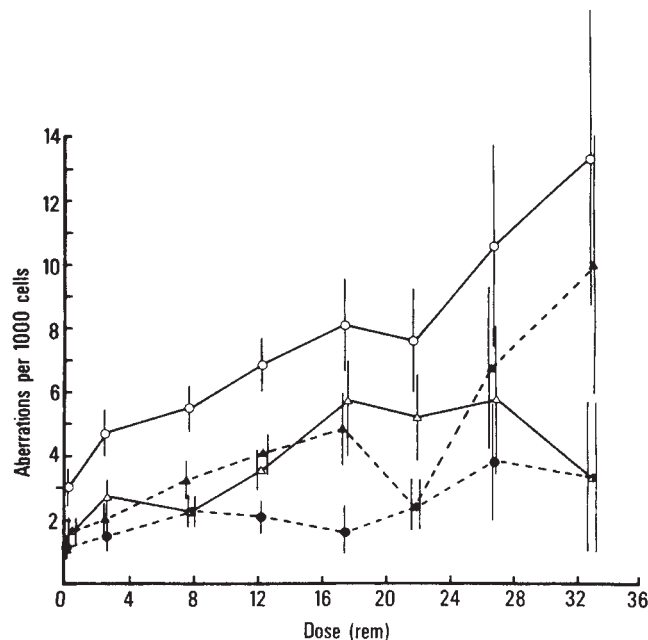


Fig. 1 Relationship between accumulated dose (rem) and mean aberration frequencies ($\pm$ s.e.m.) for dicentrics (Δ), acentric fragments ($\blacktriangle$), and for Cu ($\circ$) and Cs ($\bullet$) cells.

abnormal centromere separation (almost certainly an abnormal X chromosome^{10,11}) and chromatid aberrations, although not all these abnormalities would be expected to be consequences of radiation exposure (see below). All blood samples were coded and the cytological observer had no knowledge of the radiation history of any sample at the time of scoring.

Dose estimates (in rems) from film badges were provided by the Admiralty Radiation Records Centre, Institute of Naval Medicine, and were obtained independently of the receipt of blood samples and some time after completion of chromosome analyses. The radiation sources were nuclear submarine reactors undergoing refit and emitting mixed neutron- γ radiation, but the exposures were stated to involve 'almost exclusively gamma radiation'.

The data obtained over the 10-yr period were grouped by cumulative dose into 5-rem intervals and the basic data from the study are presented in Table 1 and Fig. 1. From Fig. 1 it seems that the incidence of cells containing all types of structural chromosome aberrations is positively correlated with dose. However, we have evidence from studies on a variety of populations for an increase in the spontaneous frequency of aberrations—in the absence of known radiation exposure—with increasing age (unpublished data and see also ref. 12). Also, by

Table 1 Frequencies of chromosome abnormalities in the various exposure groups

Dose range (rem)	0.0–0.9	1.0–4.9	5.0–9.9	10.0–14.9	15.0–19.9	20.0–24.9	25.0–29.9	≥ 30.0	Total
No. of cultures*	87	85	91	91	36	29	11	6	436
No. of individuals†	80	80	71	68	29	22	9	6	197
Total cells examined	8,700	8,500	9,200	9,075	3,700	2,900	1,040	600	43,715
Mean age (yr)	32.6	31.7	37.4	39.8	39.4	41.3	42.3	41.3	36.4
Mean dose (rem)	0.2	2.5	7.7	12.2	17.4	21.8	26.7	32.9	8.7
Mean date of culture	1971.9	1971.7	1972.1	1973.0	1973.8	1974.9	1975.0	1975.2	1972.6
Cells with additional or missing monocentrics (aneuploids)	219	247	317	305	105	98	24	13	1,328
Cells with abnormal monocentrics (Cs cells)	10	13	21	19	6	7	4	2	82
Cells with unstable aberrations (Cu cells)	26	40	50	62	30	22	11	8	249
No. of acentric elements	14	17	30	37	18	7	7	6	136
No. of dicentrics	14	23	21	32	21	15	6	2	134
No. of rings	2	6	5	4	0	3	1	0	21
No. of chromatid aberrations	156	200	199	224	73	63	30	21	966
Abnormal medium-sized (X?) chromosomes	20	7	18	29	14	8	4	2	102

* Few workers received as much as 5 rem per year so that more than one culture from an individual may occur within a given dose group.

† Approximately half of the individuals had two or more cultures analysed.

Table 2 Results of multiple linear regression analysis for aberrations showing a significant dose dependence

Type of aberration	Incidence per 1,000 cells†	Increase in incidence per cell/rem $\times 10^4$ of 'total' dose‡	t	Increase in incidence per cell/rem $\times 10^4$ of 'early' dose‡	t_1	Increase in incidence per cell/rem $\times 10^4$ of 'recent' dose‡	t_2
Acentric elements	1.77(± 0.42)	1.55(± 0.38)	4.1***	1.20(± 0.52)	2.3*	2.32(± 0.91)	2.5*
Dicentrics	1.85(± 0.35)	1.40(± 0.38)	3.7***	1.01(± 0.53)	1.9	2.32(± 1.01)	2.3*
Acentric elements, dicentrics and rings combined	4.06(± 0.61)	2.99(± 0.56)	5.4***	2.39(± 0.76)	3.1**	4.38(± 1.40)	3.1**

† Baseline incidence is calculated at zero dose, mean age (36.4 yr) and mean date of culture (1972.6).

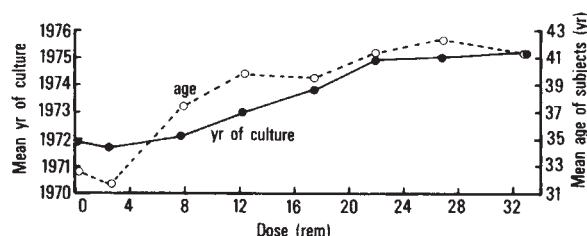
‡ These are equivalent to the coefficients b , b_1 and b_2 of the model described in the text.

*, **, ***, Significance levels at 0.05, 0.01 and 0.001 respectively.

the very nature of the dose accumulation process, it is to be expected that older workers and samples from later years would contribute disproportionately to the higher dose categories. The positive correlation between dose and age, and dose and year of culture, and aberration frequency is shown in Fig. 2 and it is clearly necessary to take account of the influence of these two factors in examining for correlations between level of exposure and aberration yield. For this reason the data were initially analysed by multiple linear regression under the assumption that n_i , the number of aberrations in the i -th culture, is Poisson-distributed with mean μ_i given by

$$\mu_i = N_i(a + bx_i + cy_i + dz_i)$$

where N_i = number of cells examined in i -th culture; x_i = cumulative dose received by the blood donor at the time of the i -th culture; y_i = age of donor at the time of culture; and z_i = year in which sample for the i -th culture was taken. We accept the conventional assumption of a Poisson distribution, but note that the small numbers of aberrations give insufficient data for a rigorous test of this premise. The parameters were estimated by maximising the likelihood, the significance of an effect being judged by standard likelihood-ratio tests. In this way a 'best linear model' containing only the significant ($P \leq 0.05$) effects was found for each type of aberration. Those aberrations whose 'best linear model' included a dose effect were then further analysed in terms of: (1) a model with a term x_i^2 to see whether significant second-order effects of dose were present; (2) a model with a term $x_i y_i$ added to see whether a significant age-dose interaction was present.

**Fig. 2** The relationships between accumulated dose (rem) and year of culture (●), and dose and age of blood donor (○).

Studies on radiotherapy patients and on individuals accidentally exposed to high doses of radiation have shown that the frequency of unstable aberrations in peripheral blood lymphocytes, such as dicentrics, rings and fragments, decreases in the months and years following exposure¹³. Thus, for a given time of blood sampling, an exposure a few days or weeks before sampling results in a higher detected incidence of induced unstable aberrations than an equivalent exposure received a year or more before sampling. For this reason a third model with terms $b_1 x_{1i} + b_2 x_{2i}$ replacing bx_i in the best linear model was also considered, where x_{1i} = cumulative dose received before the beginning of the second full calendar year preceding the date of culture, and $x_{2i} = x_i - x_{1i}$ = the dose received since that time. The aim of this third approach was therefore to establish whether the frequency of a given aberration type showed a stronger dependence on the dose received recently (x_{2i}) than on that received earlier (x_{1i}), as would be expected if there were a genuine dose-effect causal relationship.

Dependence of aberration incidence on dose, age and time of exposure

These analyses to take into account the contribution of age and date of culture revealed significant effects of dose for the incidence of dicentric aberrations, acentric fragments and cells with symmetrical rearrangements (Cs cells); these data are summarised in Table 2. *In vitro* radiation 'model' experiments and 'high dose' *in vivo* studies have clearly shown that the incidence of dicentric ($\pm$ ring) aberrations, acentric fragments and total cells with such unstable aberrations (Cu cells) is dose dependent and that these aberrations are typical radiation-induced changes: the difficulty of detecting symmetrical rearrangements contributes to the much lower incidence of Cs relative to Cu cells and to the diminished dose dependence of Cs cell types. In contrast, as most peripheral blood lymphocytes are in a G1 phase when exposed to radiation, the majority of chromatid-type aberrations observed in such cells are not radiation-induced and these aberrations, and the incidence of aneuploidy in first post-irradiation mitoses, show little dependence on dose. Similarly, the incidence of a medium-sized chromosome with abnormally separated chromatids is independent of radiation exposure, but is heavily dependent on age and sex, being prevalent in ageing females and identified as an X chromosome^{10,11}. In the present data a significant age-effect relationship, but no dose-effect relationship, was found for aneuploid cells, for chromatid aberrations and for 'X chromosomes' with abnormal centromere separation, so that these results are as expected.

The above analyses suggest that the asymmetrical, or unstable, aberrations observed in the blood cells of these dockyard workers are a consequence of their exposure to radiation. Detailed analysis of these aberrations further reveals that: (1) there are no detectable second-order effects of dose—all data are consistent with a linear dose response; (2) in all categories there is a positive age-dose interaction (Table 3), indicating a greater susceptibility to radiation-induced chromosome damage with increasing age—as suggested by earlier *in vitro* studies^{14,15}; this was statistically significant ($P < 0.05$) for dicentric aberrations; and (3) for all categories the dependence on 'recent' dose is greater than on 'early' dose, although in no case do the coefficients b_1 and b_2 differ significantly (Table 2).

Implications of the findings

The demonstration of a significant dose-dependent increase in chromosome aberrations in peripheral blood leukocyte chromosomes in a population of closely monitored nuclear-dockyard workers, subject to occupational radiation exposure

Table 3 Age-dose interactions for aberrations with a significant dose-dependence

	Significance level of interaction effect	Estimated increase in incidence per rem per cell ($\times 10^4$)		
		Age 25 yr	Age 40 yr	Age 55 yr
Acentric elements	$P = 0.24$	1.04(± 0.59)	1.64(± 0.40)	2.25(± 0.71)
Dicentrics	$P = 0.030$	0.53(± 0.59)	1.57(± 0.38)	2.60(± 0.68)
Acentric elements, dicentrics and rings combined	$P = 0.021$	1.57(± 0.85)	3.27(± 0.57)	4.97(± 0.99)

within the maximum permissible level of 5 rem per yr, raises a number of questions. We should perhaps first note that the estimated rate of increase of dicentric aberrations with dose (1.4×10^{-4} dicentrics per cell per rem) is of a similar order to that obtained from our earlier data⁸ on a population of occupationally exposed personnel at UKAEA Windscale, although the number of cells examined in the Windscale study was insufficient to establish significance. In this context it should be noted that the effect of increasing dose is not seen in successive cultures from the same individual, at the exposure levels encountered by our population, because the number of cells examined per individual is insufficient. For example, in these conditions of exposure, the detection of the estimated effect of 10 rem on the incidence of dicentrics in a single individual would necessitate examining of the order of 10,000 cells—a formidable task.

The yield of dicentric aberrations, or of acentric fragments, in cells from individuals obtained before occupational exposure, or after exposure to <1 rem, was ~1 in 700 cells, rising approximately fourfold after accumulation of doses of 20 to 30 rem. The observed increase is therefore not large, but it is a direct expression of increased genetic damage caused by radiation exposure. The dicentric aberrations observed are frequently cell lethal, as also are a proportion of the free fragments, but some of the cells containing acentric fragments, and those with symmetrical rearrangements such as translocations, are viable and a proportion of these are detected as Cs cells. Cs cells are not easily distinguished, represent ~1 in 900 cells studied in individuals receiving <1 rem, and are approximately three times as frequent in individuals who accumulated more than 25 rem. It is not clear whether there is a somatic effect on the individual as a result of the presence of Cs cells, although each such cell presumably contains some kind of mutational change and there are many thousands of such cells present in blood lymphocytes and bone-marrow cells of individuals not exposed to radiation. Our data therefore tell us nothing about possible biological consequences, but merely demonstrate that it is possible to detect a biological effect at the chromosome level of occupational exposure to ionising radiation below the internationally agreed maximum permissible levels.

There has been much recent publicity concerning possible increased cancer incidence—particularly leukaemias and lymphomas—in occupationally exposed personnel in the USA in a Naval dockyard^{16,17} and in the atomic energy plant at Hanford^{18,19}. However, the data on which some of these claims have been based, and the analyses involved, have been very heavily criticised^{20–23}. It is therefore pertinent for us to point out that the exposed population that we have studied is small, subject to very low levels of radiation exposure for periods up to

only 10 yr—and is therefore unlikely to provide useful data on the incidence of malignant disease. During the 10-yr study there were 2 deaths amongst the 197 men studied, both due to myocardial infarction: there was no record of deaths due to leukaemia or other malignant disease. Examination of the Registrar General's returns for deaths attributed to, or associated with, leukaemias for the area which includes our naval dockyard, has revealed no increase in the incidence of leukaemia relative to other populations over the years 1967–77, and no disproportionate number of dockyard workers suffering such deaths. We emphasise again, however, that the population size is small and it would seem sensible to monitor causes of death in a larger population of workers exposed to radiations within the occupational limits—a project that has already been initiated by the National Radiological Protection Board²⁴.

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Selective activation of human β - but not γ -globin gene in human fibroblast $\times$ mouse erythroleukaemia cell hybrids

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The human α - and β -globin genes have been activated in MEL $\times$ human fibroblast cell hybrids. However, even though the human γ - and β -globin genes are closely linked and were shown in these hybrid clones to be present in approximately equal numbers, no human γ -globin mRNA was produced. Thus, the human β - and γ -globin genes in these cells are differentially regulated apparently by a positive regulatory factor(s) specific for individual globin genes.

IN order to define the mechanism of globin gene regulation in mammalian cells, we have examined the expression of the mouse and human haemoglobin genes in somatic cell hybrids between mouse erythroleukaemia (MEL) cells and various human cell lines^{1–6}. Previous studies have shown that fusions between MEL cells and human (or Chinese hamster) erythroid cells resulted in co-expression of mouse and human (or Chinese hamster) haemoglobin^{1,2}; fusions of MEL cells with non-erythroid cells resulted in hybrids which could not be induced to make haemoglobin^{3,4,7–10}, even though the globin genes were present in the hybrid cells³. Gopalakrishnan *et al.*⁴ obtained evidence for the existence, in the cytoplasm of non-erythroid cells, of a

Table 1 Phenotype of 2S MEL × HLN hybrids and parents

Cell line	Chromosomes			Isozymes				
			%	LDH			APRT	
	Mean	Range	Meta-centrics	M	H-A	H-B	M	H
Parents								
2S MEL	57.3	44–75	10.3	+++	0	0	+++	0
HLN	46.1	44–48	88.6	0	+++	+++	0	+++
Hybrids								
XVIII-5	68.0	51–86	17.1	+++	++	+++	+++	0
XVIII-15	55.9	42–74	11.5	+++	+++	0	+++	0
XIX-2	122.3	73–159	22.2	+++	+	+++	+++	±
XIX-7	72.1	60–89	20.3	+++	++	0	+++	0
XIX-10	74.4	59–90	24.4	+++	+	0	+++	±
XX-8	66.4	46–84	13.7	+++	++	++	+++	0

2S MEL is an MEL cell line containing a sub-tetraploid complement of chromosomes⁵; it is TK⁻. HLN stands for a human Lesch-Nyhan skin fibroblast primary cell line (CRL 1112, ATCC), and is HGPRT⁻. XVIII-5 is the 5th clone of the 18th fusion of 2S MEL cells to human non-erythroid cells. For the chromosome analysis: approximately 10⁷ cells were washed in Dulbecco's phosphate-buffered saline, swollen in 0.075 M KCl, fixed in 3:1 methanol-acetic acid and dropped onto glass slides. Photographs of at least 15 metaphase spreads per clone were counted to estimate the total number of chromosomes and per cent of metacentric chromosomes. The spreads were made from an aliquot of the same expanded and induced cell population from which the isozyme analyses were done and from which the RNA and DNA were isolated for the studies in Table 2 and Figures 2–4. For the isozyme analysis: human chromosome 11 is known to have LDH-A and the γ - δ - β globin gene complex²⁰; human chromosome 16 is known to have APRT and human α -globin¹⁹; LDH-B is on human chromosome 12. APRT analyses were by the method of Tischfield *et al.*³⁰. Only hybrids positive for human LDH-A (chromosome 11) are shown; approximately 25% of the successful fusions between 2S MEL and HLN cells resulted in retention of chromosome 11 after expansion. M, mouse; H-A, human LDH-A; H-B, human LDH-B; H, human. The scale is: 0, None detectable; ± → +++ = increasingly positive.

negative regulatory factor which prevented the subsequent induction of haemoglobin in MEL 'cybrids' obtained by fusion of intact MEL cells with enucleated non-erythroid cells. Recently, Deisseroth and Hendrick¹¹ showed that human α -globin mRNA as well as mouse globin mRNA was expressed in 1S MEL × human erythroid cell hybrids when human chromosome 16 had been selectively retained. It is not known if a fibroblast α -globin gene would have been activated in these 1S MEL hybrids.

We wish to learn if erythroid cells (such as MEL cells) might contain one or more positive regulatory factors. Evidence for positive regulation would exist if conditions could be found that result in activation of fibroblast globin genes (as well as MEL globin genes) in MEL × fibroblast hybrids. A gene dosage effect has been used by others to obtain phenotypic expression of differentiated functions in somatic cell hybrids^{12–17}; an increased input of chromosomes from one parental cell can yield hybrid cells in which a previously unexpressed function might be activated^{17,18}. Weiss and coworkers¹⁷ have extensively studied the activation of albumin synthesis and other liver functions in rat hepatoma × mouse lymphoid cell hybrids. The expression of rat hepatic functions, and the activation of such functions from the mouse lymphoid chromosomes, was greatly increased by the use of a 2S (tetraploid) hepatoma parental cell¹⁷. Our laboratory developed a stable 2S (tetraploid) MEL cell line⁵ and demonstrated that 2S MEL × fibroblast hybrids were inducible for haemoglobin as shown by positive benzidine staining of hybrid cell clones. It was also demonstrated that 1S MEL × fibroblast fusions can result in haemoglobin inducible hybrids although the efficiency is greatly increased when 2S MEL cells are used.

We have now tested for the expression of mouse and human globin genes by measuring the amounts of mouse and human globin mRNA in somatic cell hybrids derived from fusions of 2S MEL cells with human fibroblasts. The human α -globin gene is on chromosome 16 (ref. 19) and the human γ - δ - β globin gene locus is on chromosome 11 (ref. 20). Adults synthesise primarily haemoglobin A ($\alpha_2\beta_2$) while the human fetus synthesises primarily haemoglobin F ($\alpha_2\gamma_2$). Soon after birth, haemoglobin F is replaced by haemoglobin A. Since the γ - and β -globin genes are

closely linked, our experimental model is unique in that it provides the opportunity to examine the expression of two closely linked genes at the molecular (mRNA) level. Our results indicate that, when human chromosome 16 is present in the hybrid clone, human α -globin mRNA is produced (in addition to mouse globin mRNA); when chromosome 11 is present, human β -globin mRNA is found. Surprisingly, however, even when human β -globin mRNA is present in large amounts, no human γ -globin mRNA is detectable. Thus, even though the β - and γ -globin genes are closely linked and were shown to be present in approximately equal numbers in these hybrid cells, only the β -globin gene is expressed. Thus, the mechanism of differential regulation of human β - and γ -globin genes seems to involve, at least in these cells, a globin gene specific regulatory factor(s).

Hybrids between MEL cells and human fibroblasts

Fusions were performed between thymidine kinase deficient (TK⁻) 2S MEL cells⁵ and various hypoxanthine-guanine-phosphoribosyl-transferase deficient (HGPRT⁻) human non-erythroid cell lines. In each fusion, several individual clones were grown to 10⁶ cells and analysed for human lactate dehydrogenase-A (LDH-A); human chromosome 11 contains LDH-A and the γ - δ - β globin gene complex. Positive clones were expanded to 5 × 10⁸ cells and a repeat isozyme analysis carried out. If the clone was still positive for LDH-A, a portion of the

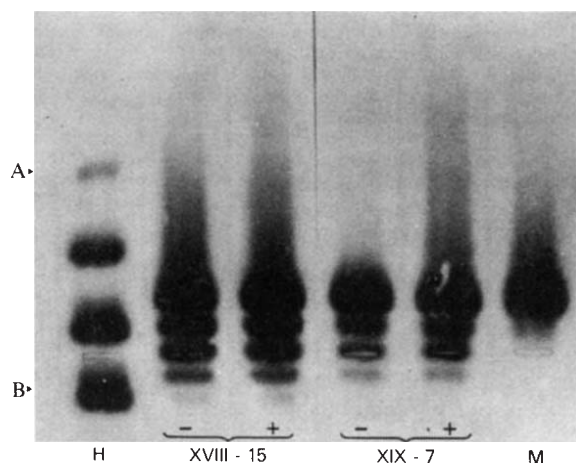
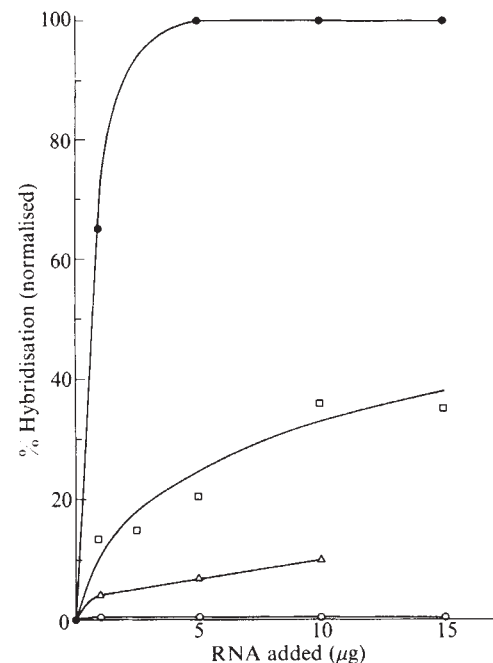


Fig. 1 Lactate dehydrogenase (LDH) isozyme pattern of the parental 2S MEL cells, human Lesch-Nyhan (HLN) skin fibroblast primary cells, and two of the hybrids between them. The parental lines, 2S MEL and human Lesch-Nyhan fibroblasts (see Table 1), were grown in F14 medium (available without NaHCO₃ from Gibco as F12-modified medium 72004) supplemented with 3.7 g l⁻¹ NaHCO₃ and with 10% fetal calf serum³¹. 2S MEL cells (1 × 10⁷) were mixed with 1 × 10⁶ trypsinised HLN fibroblasts, washed twice in serum-free medium, resuspended in 1 ml of serum-free medium containing 250 HAU of inactivated Sendai virus, incubated at 0 °C for 15 min, then at 37 °C for 1 h, resuspended at 5 × 10⁴ cells ml⁻¹ in medium with serum, and incubated at 37 °C in 10% CO₂ at 100% humidity⁵. The next day cells were plated in 0.1 ml in microtiter wells (Falcon) at different dilutions. After 24 h, 0.1 ml of medium containing 2 × HAT was added to each well. After 2–3 weeks, wells containing visible colonies (all floating cells) were transferred to 60-mm dishes in medium containing 10⁻⁴ M hypoxanthine and 1.6 × 10⁻⁵ M thymidine. After 1 week in this medium, the cells were transferred to regular growth medium (F14 plus 10% fetal calf serum) and expanded to 1 × 10⁶ cells. Each clone was then analysed for human LDH-A. Electrophoresis was carried out using the Gelman LDH isozyme electrophoresis system (according to Technical Bulletin 23) with Corning AC1 agarose electrophoresis film. Positive clones were grown to 3–5 × 10⁸ cells and re-checked for LDH-A. One-half the culture was transferred to medium containing 2.0% DMSO and incubated for 5–6 days (reaching approximately 10⁹ cells); the remaining half was continued in regular growth medium for 2–4 days to reach 10⁹ cells. Aliquots from each incubation were taken for LDH and APRT isozyme electrophoresis, chromosome analysis, benzidine reactivity and ³H-leucine incorporation into globin chains. All remaining cells were used for RNA and DNA extraction as described in the legend to Fig. 2. H, human Lesch-Nyhan fibroblasts; M, 2S MEL cells; XVIII-15 and XIX-7 are hybrids; -, means after expansion to 10⁹ cells but without induction; +, means after expansion and induction of haemoglobin by 5–6 days growth in 2% dimethylsulfoxide. A, human LDH-A tetramer; B, human LDH-B tetramer.

Fig. 2 Hybridisation of hybrid cell RNA to ^3H -mouse globin cDNA and ^{32}P -human α -globin cDNA. Hybrid cells (see Fig. 1 legend) were pelleted, washed once in minimal essential medium, repelleted, and then suspended in a solution of 10 mM Tris-HCl, pH 8, 2 mM MgCl_2 , 3 mM CaCl_2 , 0.1% Triton X-100, 0.5 mM dithiothreitol, and 0.1 M sucrose. Nuclei were obtained by homogenisation with a motor driven Teflon-glass Dounce homogeniser (4–6 strokes at full speed); the nuclear suspension was diluted 1:1 in an identical buffer but with a sucrose concentration of 0.25 M. 20 ml of the nuclei suspension was then layered onto 10 ml of a 0.33 M sucrose cushion containing 10 mM Tris-HCl, pH 8, 5 mM MgCl_2 , and 0.5 mM dithiothreitol. The nuclei were pelleted by centrifugation at 800 g (2,200 r.p.m. in a GLC tabletop centrifuge) for 5–10 min. The supernatant fraction was removed for RNA extraction; DNA was extracted from the nuclear pellet as previously described²⁶. For RNA extraction, the supernatant from the preparation of nuclei was made 0.1 M in NaOAc (pH 5) and 10 mM EDTA. Subsequent phenol extraction at 55 °C, ethanol precipitation, DNase treatment, re-extraction with phenol, and passage over a G-50 Sephadex column was as described previously^{32,33}. Mouse globin cDNA was prepared as previously described³⁴ using mouse reticulocyte mRNA prepared from polysomes³² as a template for RNA-directed DNA polymerase. ^3H -CTP was used as the label yielding a probe with a specific activity of 10,000–12,000 c.p.m. μg^{-1} . A size fraction 500–600 nucleotides in length was selected by alkaline sucrose gradient centrifugation³⁴. Plasmid DNA (100–150 μg) from JW 101 (human α), JW 102 (human β), or JW 151 (human γ)²¹ was digested with *Mbo*II (JW 101) or *Hha*I (JW 102, JW 151) and the resulting DNA fragments were fractionated on a sucrose gradient (5–20%) in 10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA³⁵. The largest fragment, which contains the globin gene insert, was recovered by ethanol precipitation. Nick-translation^{36,37} in the presence of ^{32}P -dCTP resulted in a specific activity of approximately 10^8 c.p.m. μg^{-1} . Each plasmid fragment was annealed in 50% formamide, 0.5 M NaCl to homologous mRNA (that is, the fragment containing either the α - or β -globin gene was annealed to human adult globin mRNA (α and β) while the *Hha*I fragment containing the γ -globin gene was annealed to fetal reticulocyte mRNA (α , β , and γ) at 66.5 °C. At this temperature DNA will not reanneal, but a duplex between mRNA and the cDNA strand of the insert is formed. Digestion with *S*₁ nuclease resulted in destruction of all DNA except that in a duplex. The single-stranded cDNA fragment was then recovered by alkaline sucrose gradient centrifugation³⁵ and used for mRNA · cDNA (and DNA · cDNA) hybridisations. mRNA · cDNA hybridisation was carried out as previously described³⁴ except that both ^3H -mouse globin cDNA and ^{32}P -human α -globin cDNA were used in each double labelled reaction. Titrations were performed in 50% formamide, 0.5 M NaCl at 66.5 °C. Incubation to achieve equilibrium was for 48 h after which duplex formation was analysed by *S*₁ nuclease resistance³³. Each 10 μl reaction mixture contained 2,000 c.p.m. of ^3H -mouse globin cDNA and 2,000 c.p.m. of ^{32}P -human α -globin probe. Control incubations showed: (1) duplex formation resulting from titration with mouse reticulocyte mRNA reached 82% for the ^3H -mouse cDNA, but less than 1% for the ^{32}P -human α -cDNA; and (2), duplex formation resulting from titration with human reticulocyte mRNA reached 71% for the ^{32}P -human α -cDNA but less than 1% for the ^3H -mouse cDNA. Values were normalised by dividing each ^3H value by 0.82 and each ^{32}P value by 0.71. The ^3H values for all six hybrids were similar and were, therefore, averaged. Similar computations were performed for the data in Figs 3 and 4. ●, Hybridisation with ^3H -mouse globin cDNA (the values for all six hybrid cell RNAs have been averaged and normalized; see above); □, hybridisation of the RNA from hybrid XIX-2 with ^{32}P -human α -globin cDNA; △, RNA from XIX-10 with ^{32}P -human α -globin cDNA; ○, RNA from XVIII-5, XVIII-15, XIX-7, and XX-8 with ^{32}P -human α -globin cDNA.



cells were grown in 2% dimethylsulphoxide (DMSO) for 5–6 days and the remainder for a shorter period without inducer (see Fig. 1 legend). Aliquots from each incubation were taken for isozyme analysis, karyology, haemoglobin inducibility and ^3H -leucine incorporation into globin chains. RNA and DNA were then extracted from the remaining cells as described in Fig. 2 legend.

The phenotypic characteristics of the 2S MEL and the human Lesch-Nyhan (HLN) skin fibroblast primary parents, as well as of six hybrids between them, are shown in Table 1. Only the six hybrids which are positive for LDH-A (Fig. 1) are shown, since β -globin mRNA was not found in the hybrid clones lacking LDH-A (data not shown). APRT was examined as it is a marker for chromosome 16 which carries the human α -globin gene. Chromosome analysis shows that the hybrids possess an increased number of chromosomes and a higher percentage of metacentrics than the 2S MEL parent. All six were positive for mouse LDH, mouse APRT, and human LDH-A; three were positive for LDH-B (carried on chromosome 12); two for human APRT. XIX-2, the hybrid with the highest number of chromosomes (122 with 22% metacentrics), was the only clone positive for all three human enzymes: LDH-A, LDH-B, and APRT. As shown in Table 2, all six hybrids could be induced to make haemoglobin as determined by benzidine staining.

Detection of human globin mRNA in cell hybrids

Pure single stranded probes complementary to human α -, β -, or γ -globin mRNA were prepared from recombinant cDNA plasmid clones JW 101, 102, or 151 (ref. 21), respectively, as described in Fig. 2 legend. The cytoplasmic RNA from each hybrid line was separately hybridised to each ^{32}P -labelled human probe in the presence of ^3H -labelled mouse globin cDNA. In the data in Figs 2–4, the ^3H -mouse globin cDNA

hybridisation values for all six hybrid cell RNAs were averaged (solid circles), and all points were normalised, as described in Fig. 2 legend. Thus, the plots of α , β , and γ human globin mRNA for each of the six hybrids can be compared to each other.

As shown in Fig. 2, two hybrids contain small amounts of human α -globin mRNA: XIX-2 and XIX-10. Both these hybrids were positive for human APRT on enzyme analysis (Table 1). Five of the six hybrids positive for human LDH-A had clearly detectable human β -globin mRNA (Fig. 3). XX-8, the hybrid with the highest concentration of human β -globin mRNA and a strong LDH-A band, had 35% as many human β -globin mRNA sequences as mouse globin mRNA sequences. There is a fairly close correlation between the amount of LDH-A in a given hybrid cell line (Table 1) and the amount of human β -globin mRNA present (Fig. 3, Table 2).

None of the hybrids had detectable human γ -globin mRNA sequences, even at 60 μg RNA input (Fig. 4). When the human γ -globin cDNA probe was annealed to mRNA isolated from fetal cord blood in a simultaneous experiment, 72% of the γ probe was incorporated into duplexes; a saturation plateau was achieved above an input of 5 ng of partially purified mRNA (data not shown). As expected, since the γ - and β -globin genes are closely linked, DNA · cDNA hybridisation demonstrated approximately equal concentrations of γ - and β -globin gene sequences in the hybrid cell DNA (data not shown). Analysis of the RNA from the expanded but uninduced hybrid cell showed very low levels of mouse globin mRNA, no (or barely detectable) human β -globin mRNA, and no detectable human γ -globin mRNA.

Carboxymethylcellulose chromatography of the ^3H -leucine globin chains synthesised by the hybrids positive for human β -globin mRNA revealed a peak in the region of human β -globin. However, the calculated number of globin chains in the

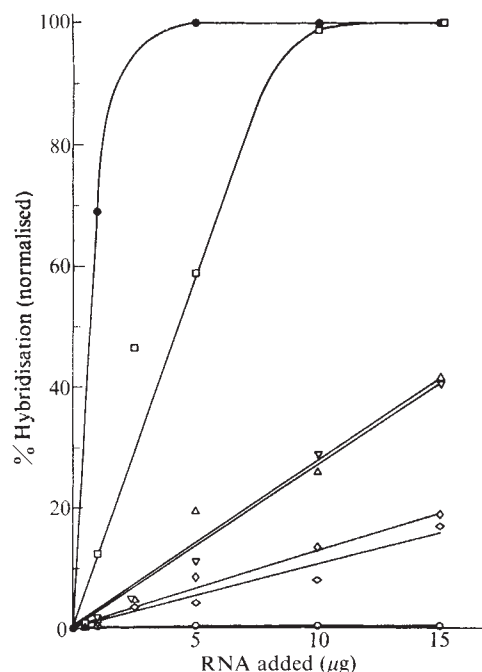


Fig. 3 Hybridisation of hybrid cell RNA to ^3H -mouse globin cDNA and ^{32}P -human β -globin cDNA. Experimental conditions were as for Fig. 2 except that human β -globin cDNA from the cDNA plasmid JW 102²¹ was used. Hybridisation of the β -globin probe to globin mRNA obtained from adult human reticulocytes reached a plateau of 78.5%. All hybrid RNA points with ^{32}P -human β cDNA were divided by 0.785 to normalise them to a maximum of 100%. ●, Average value for hybrid cell RNA with ^3H -mouse globin cDNA; open symbols are for hybrid cell RNA with ^{32}P -human β -globin cDNA; □, XX-8; △, XIX-7, ▽, XVIII-15; ◇, XIX-2; ○, XVIII-5; ○, XIX-10.

human β -globin region did not correlate with the concentration of human β -globin mRNA molecules known to be present. Further analysis of the putative human β -globin is underway.

Discussion

In this study, we have shown that the globin genes of human fibroblasts can be activated in fibroblast $\times$ 2S MEL cell hybrids. The expression of fibroblast globin genes implies the presence in the induced 2S MEL hybrid cells of a positive regulatory factor of some type. While the adult human globin genes, α and β , were consistently activated when the human chromosomes bearing these genes were present in the hybrid clones (chromosome 16 for α , chromosome 11 for β), the human γ -globin gene, which is closely linked with β -globin, was not expressed at all. Thus, in these cells, there is a differential regulation within the γ - β globin gene locus.

Several mechanisms might account for such differential gene regulation. First, a diffusible regulatory factor could act as a positive activator of the human β - but not γ -globin gene by binding to a controlling region adjacent to the globin gene structural sequences. Many examples of such regulatory factors in prokaryotes have been described²². A single factor might be responsible for activating the human α , human β , and the mouse globin genes, or several different globin gene specific factors could be involved.

A second possible mechanism which could account for differential globin gene activation in these hybrid cells would be the state of the parental cell's chromatin before and after fusion. Expression of a gene requires that its sequences be in an active conformation in nuclear chromatin. Exposure of nuclei to pancreatic DNase I results in selective digestion of active genes²³⁻²⁶. Thus, in erythroid cells, the globin genes are DNase I sensitive while in fibroblasts the globin genes are resistant. We assume that the human β - and α -globin genes were resistant in

Table 2 Haemoglobin inducibility of 2S MEL $\times$ HLN hybrids and parents

Cell line	Haemoglobin inducible (%)	Mouse globin mRNA	Human globin mRNA		
			α	β	γ
Parents					
2S MEL	60	+++	0	0	0
HLN	0	0	0	0	0
Hybrids					
XVIII-5	85	+++	0	+	0
XVIII-15	40	+++	0	++	0
XIX-2	55	+++	++	+	0
XIX-7	85	+++	0	++	0
XIX-10	70	+++	+	0	0
XX-8	95	+++	0	+++	0

Haemoglobin inducibility is the per cent of cells in the population which were benzidine positive after incubation for 5 days in 2% dimethylsulphoxide (DMSO). Actively growing floating cells were resuspended at a concentration of $0.5-1 \times 10^5$ cells/ml in F14 medium containing 10% fetal calf serum and 2% DMSO. After 5 days the cells were stained by the addition of one-tenth the volume of a fresh solution containing 0.2% (w/v) of benzidine dihydrochloride and 0.4% (v/v) H_2O_2 in 0.5 M acetic acid¹. Mouse globin mRNA was measured by hybridisation with ^3H -cDNA made from unfractionated mouse reticulocyte mRNA; human α -, β - and γ -globin mRNA were measured with pure probes obtained from recombinant cDNA plasmids (see Fig. 2 legend). The scale is: 0 = none detectable; + $\rightarrow$ +++ = increasingly positive.

the parental fibroblasts but were converted to the active conformation in the hybrid clones. It is thought that non-histone proteins may be operative in initiating and propagating the active conformation of nucleosomes associated with expressed genes. If so, these 'regulatory factors' for mouse MEL cells would act on both mouse and human α - and β -globin genes, but possibly not on the human γ -globin gene. Since adult human fibroblasts were used in this study, it is possible that, if chromatin structure of the parental cells is important, a fusion of 2S MEL cells with fetal fibroblasts would result in expression of human γ -globin mRNA. To test this hypothesis, hybrids were formed between 2S MEL and fetal amniotic cells and are now being analysed for β - and γ -globin mRNA synthesis.

Another possible mechanism of differential gene expression is selective processing of globin gene transcripts. In our present

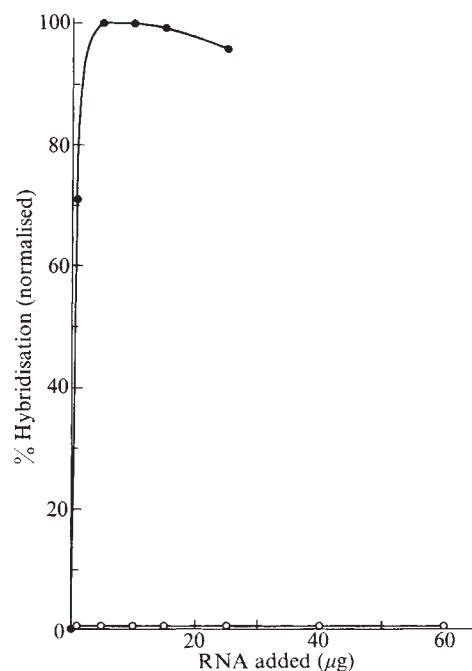


Fig. 4 Hybridisation of hybrid cell RNA to ^3H -mouse globin cDNA and ^{32}P -human γ -globin cDNA. Experimental conditions were as for Fig. 2, except that human γ -globin cDNA from the cDNA plasmid JW 151²¹ was used. Hybridisation of the γ -globin probe to globin mRNA obtained from cord blood red cells reached a plateau of 72%. ●, Average value for hybrid cell RNA with ^3H -mouse globin cDNA; ○, hybrid cell RNA with ^{32}P -human γ -globin cDNA (all six were negative).

study, the hybrid cell nuclei were used for DNA extraction, and the RNA was isolated from the cytoplasm only. It is possible that human γ -globin gene sequences were present in the nuclei, but were selectively degraded or were not processed in such a way that they could reach the cytoplasm. Thus, specific and selective MEL processing enzymes could have been involved. Extraction and analysis of nuclear RNA should detect all but rapidly degraded γ -globin gene sequences.

Whether the factor(s) that controls globin gene expression acts by binding directly to a controlling region of a globin gene 'operon', at the level of chromatin structure, by influencing processing of the primary globin gene transcript, or by some other mechanism, there is no factor in the induced MEL hybrid cell that results in expression of the human γ -globin gene.

If a diffusable positive regulatory factor(s) does exist in induced MEL cells, it should be possible to isolate and characterise it. The recent availability of cloned globin genes from genomic DNA obtained by recombinant DNA technology^{27,28} should allow cell-free systems to be developed to test the mechanism of action of these factors. As a preliminary to cell-free studies, we are attempting to isolate such putative

factors by fractionating the induced 2S MEL cells and microinjecting the cell fractions into uninduced MEL cells to look for induction^{6,29}. Preliminary results indicate that a 2S MEL cell can be induced by microinjecting 10^{-11} ml of 100 mM hexamethylene bisactamide (W. F. A. and E. Diacumakos, unpublished results). Microinjection of MEL cell fractions is now underway.

The existence of a factor specific for human β -globin implies that a similar factor might exist to control the human γ -globin gene. Such a factor might have clinical importance since the ability to express the γ -globin in β -haemoglobinopathies or in β -thalassaemia could be highly beneficial. To this end, hybrids are also being made using fibroblasts from patients (and at-risk fetuses) with sickle cell anaemia and β -thalassaemia to study the globin expression from fibroblasts of patients with beta chain abnormalities.

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Escherichia coli mutants accumulating the precursor of a secreted protein in the cytoplasm

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The maltose binding protein of Escherichia coli is secreted into the external periplasmic compartment of the cell. This article describes a selection procedure for the isolation of mutants which fail to export this protein. These mutants probably result from alterations in the amino terminal 'signal sequence', causing the maltose binding protein produced to accumulate in the cytoplasm in its precursor form.

SECRETED proteins in both bacterial and eukaryotic cells are initially synthesised as longer precursors with so-called 'signal sequences' at their amino termini, which are subsequently

excised¹⁻⁶. This signal sequence is thought to be responsible for initiating passage of the polypeptide through the membrane while it is still a nascent chain attached to the ribosome. The study of protein secretion in bacteria provides the obvious advantages of an easily-manipulated genetic system. We describe here the isolation of mutants of *Escherichia coli* in which the maltose binding protein (MBP), which is normally secreted across the cytoplasmic membrane into the periplasmic space, accumulates in the cytoplasm in its precursor form. This is most probably a result of alterations in the signal sequence. The MBP is a key component of the maltose transport system of this organism. It is a 38,000 molecular weight protein⁷ which is synthesised on ribosomes attached to the cytoplasmic side of the cytoplasmic membrane⁸. As predicted by the signal hypothesis, MBP is initially synthesised with an amino-terminal extension some 20 to 30 amino acids long⁹.

Fusions of *malE* and *lacZ* genes

To isolate mutants in the MBP (*malE* gene), we used the gene fusion technique of Casadaban^{10,11} to isolate strains in which the *lacZ* gene encoding the normally cytoplasmic enzyme β -galactosidase is fused to the *malE* gene. The product of such a fusion is often a hybrid protein in which the amino terminus of β -galactosidase has been replaced with an amino-terminal MBP sequence but which retains β -galactosidase enzyme activity. The signal hypothesis predicts that such a hybrid protein would be exported to the periplasm if it includes an intact MBP signal sequence. As described below, the properties of such fusion strains permit a direct selection for signal sequence mutants.

Strain PB72-47 is a *malE-lacZ* fusion strain which synthesises a very large hybrid protein (molecular weight 131,000) including a major amino-terminal portion of the MBP (Fig. 1). This strain does not, however, export the hybrid protein to the periplasm. Instead, it is localised to the cytoplasmic membrane (Table 1), suggesting that the export process may at least have been initiated. The strain also exhibits a rather unusual phenotype. When it is induced for the synthesis of the hybrid protein by the addition of maltose to the growth medium, cell division is inhibited concomitantly with the appearance of β -galactosidase activity. The cells at this point elongate to several times their normal length. Eventually, 3 to 4 h after induction, the cells begin to lyse. This 'maltose-sensitive' phenotype is a direct consequence of the synthesis of the hybrid protein. Mutations which eliminate the synthesis of hybrid protein (such as chain-terminating nonsense mutations in the hybrid gene) restore normal growth. The maltose-sensitive phenotype of strain PB72-47 apparently results from the cell's abortive attempt to export the hybrid proteins. For some reason, the cell's export apparatus cannot accommodate these unusually large polypeptides, and they remain literally 'stuck' in the cytoplasmic membrane. This has adverse consequences for the cell, since the failure to export these hybrid proteins also interferes with the transport of other exported proteins, which accumulate within the cell in their precursor forms¹².

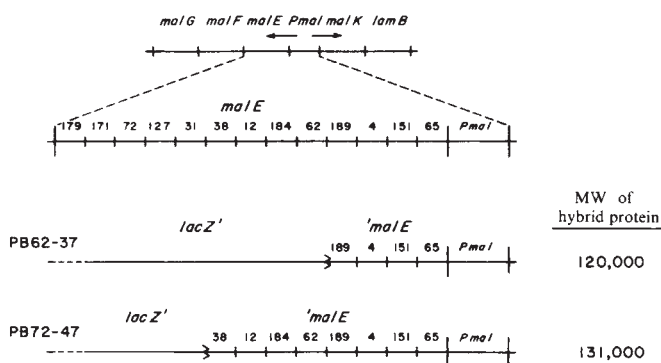


Fig. 1 The *malE* gene is one of five genes whose products are components of the maltose transport system. These are organised into two operons orientated in opposite directions and which diverge from the promoter region designated here as 'Pmal'.¹⁶ Maltose is the inducer for both operons. A deletion map of the *malEFG* operon has been constructed. The *malE* gene has been divided into 13 segments, so identified on the basis of recombination of deletions with insertions of phage Mu DNA at different points along its length (indicated here by numbers). Using these same Mu insertions, the approximate portion of the *malE* gene included in the hybrid gene in the *malE-lacZ* fusion strains PB62-37 and PB72-47 has been established. The end point within the *lacZ* gene for both fusions is genetically identical. The genetic data agrees with the molecular weights determined for the hybrid proteins encoded by these two fusion strains. Thus, the molecular weight differences discerned between different *malE-lacZ* hybrid proteins reflect differences in the contribution of the MBP to the hybrid product. Details on the construction of the deletion map, the isolation of the *malE-lacZ* fusion strains, and a complete description of the maltose-sensitive phenotype will be presented elsewhere^{18,19}.

Table 1 Localisation of β -galactosidase activity in strain PB72-47 and representative *Mal^R Lac⁺* derivatives*

Strain	β -galactosidase (Units per mg protein†)	β -galactosidase activity‡ (%)			Response to maltose
		Sonicate	Soluble	Insoluble§	
PB72-47	960	100	23	74	Sensitive
PB72-47-R1	1,483	100	71	25	Resistant
PB72-47-R2	1,275	100	65	33	Resistant

* *Mal^R Lac⁺* mutants were obtained by screening for strains that could form large, blue colonies on maltose minimal agar plates containing the indicator dye 5-bromo-4-chloro- β -D-galactoside.

† All strains were grown to mid-log phase in glycerol minimal medium at 30 °C and induced by the addition of 0.2% maltose for the final 180 min of culture. One unit of β -galactosidase activity is that amount of enzyme that will hydrolyse 1 mol of *o*-nitrophenylgalactoside per min. Note that the original fusion strains themselves are *Mal⁻* due to the inactivation of the *malE* gene. To permit induction by maltose, the hybrid genes were transferred on a λ transducing phage (see Fig. 3) into a *mal⁺* genetic background.

‡ Cells were disrupted by sonication¹¹. Enzyme activities were normalised with the activity present in the sonicate after the removal of remaining whole cells and are expressed as a percentage of such. Soluble and insoluble refer to the supernatant and pellet, respectively, after centrifugation at 100,000 g for 1 h.

§ In a separate experiment, the cytoplasmic and outer membranes were separated according to the method of Osborn *et al.*¹⁷. >90% of the membrane-associated enzyme activity fractionated with the cytoplasmic membrane.

Selection for export-defective mutants

If the attempt by the cell to export these hybrid proteins is responsible for the maltose-sensitive phenotype, then mutants resistant to maltose should include ones defective in export. Spontaneous mutant derivatives of strain PB72-47 were selected which continue to synthesise the hybrid protein but which are maltose-resistant (*Mal^R*). Since the most frequent cause of the *Mal^R* phenotype are mutations eliminating synthesis of the hybrid protein (*Mal^R Lac⁻*), only those derivatives which were *Mal^R Lac⁺* were analysed. It was reasoned that such *Mal^R Lac⁺* mutants would no longer attempt to export the protein but, rather, the protein would remain in the cytoplasm. This was so for all the *Mal^R Lac⁺* strains which we obtained (Table 1). The hybrid protein synthesised by several of these *Mal^R Lac⁺* strains seems identical in size to the hybrid protein produced by the parental strain (Fig. 2). However, since these proteins for the most part are no longer membrane-associated, they are apparently subject to proteolytic degradation, most probably at their amino-terminal end, as indicated by the appearance of a second maltose-inducible band. This second band has an apparent molecular weight nearly identical to the monomer subunit of β -galactosidase (116,000). Such degradation has been observed with other hybrid proteins containing a β -galactosidase moiety^{13,14}.

In strain PB72-47, the amino-terminal extension provided by the MBP moiety of the hybrid protein presumably directs the hybrid protein into the cytoplasmic membrane. One possible explanation for the failure of these *Mal^R* strains to attempt to export the hybrid protein is that the protein produced by these strains has an altered amino-terminal signal sequence which the cell's export apparatus cannot recognise. If this is so, then the mutation responsible for the *Mal^R* phenotype must be genetically tightly linked to the hybrid gene. Since the *malE-lacZ* fusion is carried by a λ p (plaque-forming) transducing phage, linkage information was obtained simply by isolating the λ phage from each *Mal^R Lac⁺* fusion strain and subsequently using it to lysogenise the *mal⁺ Δlac* strain MC4100. If the resultant lysogen is *Mal^R Lac⁺*, the mutation must have been cotransduced with the hybrid gene. This proved to be so for all of a number of independently-isolated *Mal^R Lac⁺* strains.

Incorporation of signal sequence mutations into the *malE* gene

If the hybrid proteins synthesised by these *Mal^R Lac⁺* strains have an altered signal sequence, the mutations responsible must be located early in the *malE* portion of the hybrid gene. We determined the effect of these mutations on the localisation of

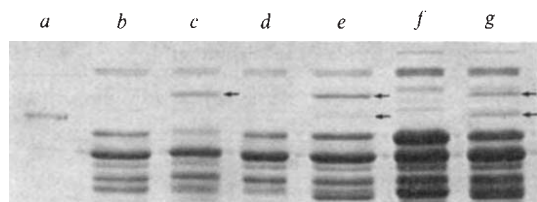


Fig. 2 SDS-polyacrylamide gel electrophoresis of whole cell extracts of *malE-lacZ* fusion strains. Whole cell extracts were prepared and electrophoresis was performed as described elsewhere²⁰. Only the relevant upper one-third portion of the gel is shown. *a*, Purified *E. coli* β -galactosidase, 2.5 μ g; *b*, strain PB72-47, uninduced; *c*, strain PB72-47, induced; *d*, strain PB72-47-R1, uninduced; *e*, strain PB72-47-R1, induced; *f*, strain PB72-47-R2, uninduced; *g*, strain PB72-47-R2, induced. The hybrid proteins are easily identified as maltose-inducible proteins of high molecular weight (arrows). In addition, *Mal*^R derivatives exhibit a second maltose-inducible band of molecular weight nearly identical to native *E. coli* β -galactosidase (arrows, see text).

the wild-type *malE* product, the MBP. As described in Fig. 3, these mutations could be recombined onto the wild-type *malE* gene. The resultant recombinants had a *Mal*⁻ phenotype when scored on maltose tetrazolium agar, indicating that their ability to utilise maltose as a carbon source had been impaired. However, it was found that these strains would grow slowly on maltose minimal medium. Two of these strains (PB1101 and PB1102), derived from independently isolated *Mal*^R *Lac*⁺ fusion strains, were characterised further. The mutant alleles were designated *malE101* and *malE102*, respectively.

To confirm that the correct mutations had been incorporated into the *malE* gene by this construction, strains PB1101 and PB1102 were lysogenised by the *Lpp* transducing phage carrying the original *malE-lacZ* fusion present in strain PB72-47. The lysogen obtained was maltose-sensitive, but *Mal*^R *Lac*⁺ derivatives were obtained at a very high frequency, a result of the reciprocal recombination event described in Fig. 3. In a

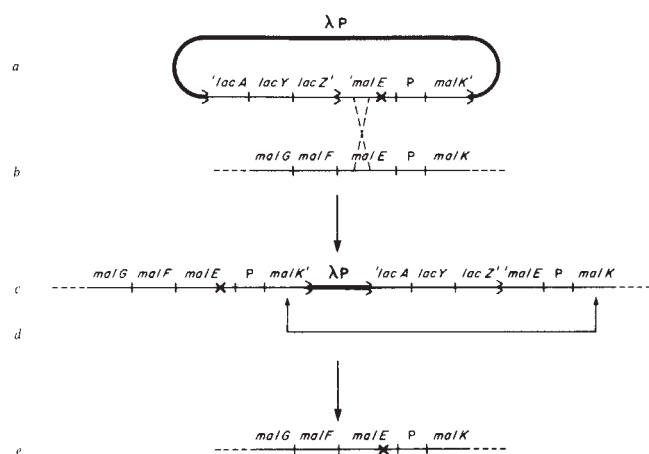


Fig. 3 Recombination of the *malE101* mutation onto a wild-type *malE* gene. *a*, the λ P transducing phage that carries the *malE-lacZ* hybrid gene from strain PB72-47-R1. The mutation *malE101* is indicated by an $\times$. *b*, A portion of the chromosome of the *mal*⁺ strain MC4100 which includes a wild-type *malE* gene. The λ P transducing phage is Δatt and strain MC4100 is Δlac . Thus, lysogenisation can most efficiently be achieved by recombination between their homologous *mal* DNA regions. Integration of the phage DNA as drawn results in a lysogen (*c*) in which the *malE101* mutation has been crossed onto an intact *malE* gene. Curing of the phage DNA by the slightly different homologous crossover indicated by the arrows (*d*) leaves the *malE101* mutation behind in the chromosome (*e*), yielding strain PB1101. Strain PB1102 was constructed in an identical manner.

similar manner, it was found that the *malE101* and *malE102* mutations could be incorporated by recombination into the hybrid gene of the maltose-sensitive fusion strain PB62-37 (again, resulting in a *Mal*^R *Lac*⁺ phenotype). Since only a small promotor-proximal portion of the *malE* gene is present in this fusion (Fig. 1), this indicates that the *malE101* and *malE102* mutations must, indeed, be located early in the *malE* gene. More precise mapping was complicated by the fact that strains PB1101 and PB1102 can still utilise maltose as a carbon source.

Identification of a mutant MBP precursor

As it appeared that there might be a defect in the export of the MBP in the mutant strains, cell extracts of strains PB1101 and PB1102 were examined for the form and location of the mutant proteins. Whole cell extracts were prepared from cultures that had been grown on glycerol (uninduced) or maltose (induced) and examined on SDS-polyacrylamide gels (Fig. 4). The maltose-inducible band corresponding to wild-type MBP is not present with either strain. However, a new maltose-inducible band is discerned which migrates with a molecular weight some two to three thousand larger than native MBP. This protein is very similar in size to that reported for the MBP precursor made *in vitro*⁹.

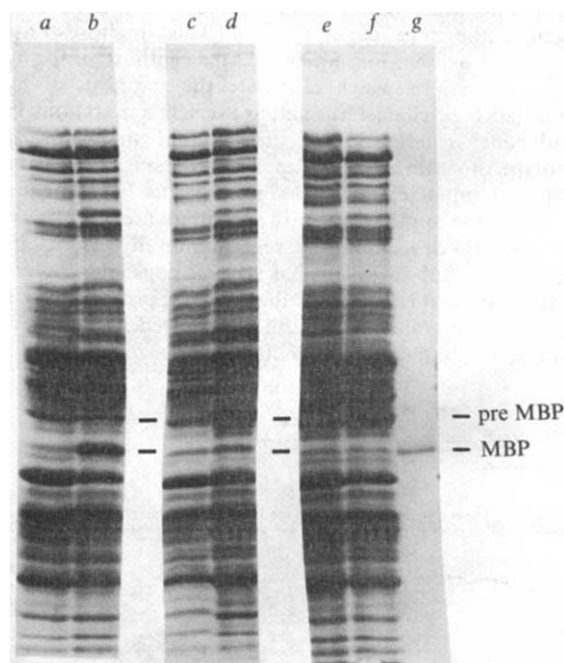


Fig. 4 SDS-polyacrylamide gel electrophoresis of whole cell extracts of *malE* mutant strains. Whole cell extracts were prepared and electrophoresis was performed as described elsewhere²⁰, except that the acrylamide concentration here is 10%. *a*, Strain MC4100, uninduced; *b*, strain MC4100, induced; *c*, strain PB1101, uninduced; *d*, strain PB1101, induced; *e*, strain PB1102, uninduced; *f*, strain PB1102, induced; *g*, purified *E. coli* native MBP, 2.0 μ g. The position of native MBP in the *mal*⁺ strain MC4100 and the position of the presumed MBP precursor (preMBP) in the two mutant strains are indicated. Everything shown in the figure is from the same slab gel.

The simplest explanation for these results is that strains PB1101 and PB1102 produce a MBP with an altered amino-terminal signal sequence. This sequence is not recognised by the cell's export apparatus and the protein accumulates in the cytoplasm in its larger (unprocessed) form. Different cell fractions of strains PB1101 and PB1102 were prepared and run on SDS gels, in order to determine the cellular location of this new maltose-inducible protein. A maltose-inducible protein which is larger than native MBP is observed in soluble extracts from both strains. This protein is somewhat smaller than the presumed precursor observed in whole cells and is believed to correspond

to a breakdown product of the precursor. Only minute amounts of this protein are released by the cell by a cold osmotic shock procedure which is used to operationally define periplasmic proteins in Gram-negative bacteria¹⁵. Therefore, this protein must be primarily located in the cytoplasm.

In order to verify that the presumed precursors observed in the mutant strains are, indeed, related to the MBP, antiserum prepared against native *E. coli* MBP was used to immune precipitate the soluble extract from a maltose-grown culture of strain PB1101 labelled with ¹⁴C-amino acids (Fig. 5). The new maltose-inducible protein observed by gel electrophoresis of the soluble extract of strain PB1101 was specifically precipitated by this antiserum. In addition, several minor proteins in the same molecular weight range were precipitated from the mutant extract. Since none of these proteins was precipitated when the antiserum was pre-incubated with purified, unlabelled MBP, it was concluded that all of the proteins precipitated were antigenically related to MBP. One of the minor proteins so precipitated ran on the gel with an apparent molecular weight identical to native MBP. This may represent a small amount of MBP that was exported to the periplasm in the mutant strain and correctly processed, allowing strain PB1101 to utilise maltose as a carbon source, albeit inefficiently. Additionally, a minor protein so precipitated from the mutant strain ran with the same apparent molecular weight as the presumed precursor observed in whole cells (see Fig. 4). We conclude that this protein is the

MBP precursor, and that it is fairly stable in whole cells. However, when the cells are disrupted under non-denaturing conditions, this protein is subjected to some proteolytic degradation, and very little remains intact even though all manipulations are performed at 4°C. Presumably, the enzyme(s) responsible for this proteolytic degradation are located in the periplasm or outer membrane where they are not accessible to the mutant MBP precursor until the cells are broken. However, these do not necessarily have any relationship to the enzyme(s) responsible for processing normal exported proteins to their mature sizes.

Reversion analysis

For both strains PB1101 and PB1102, we obtained *mal*⁺ revertants by growing these strains in maltose minimal liquid medium through many generations. Since *mal*⁺ revertants had a considerable selective advantage in such conditions, with time they accumulated to form the majority class of the population. Several independently-isolated revertants from both strains were characterised. In all cases, these appeared to be 'true' revertants, in that the MBP synthesised by these strains is made in normal amounts and found in the periplasm in its native form. That strains PB1101 and PB1102 spontaneously revert to wild-type *mal*⁺ indicates that the mutations *malE101* and *malE102* result in single amino acid substitutions in the MBP polypeptide.

Discussion

Complete or partial amino acid sequences for the amino-terminal extension of a number of exported proteins have been reported (summarised by Habener *et al.*¹⁶). Most of these are from eukaryotes. All these sequences contain a central region of some 10 to 12 non-polar and hydrophobic amino acid residues, although it is a different sequence in each case. There is no other feature common to these primary structures which readily identifies itself as being key to its recognition as a signal initiating protein export. We have now isolated *E. coli* strains which fail to secrete the MBP, most probably resulting from mutational alterations in the signal sequence region of this protein. We are now determining the precise nature of the alterations, in order to define that feature of the signal sequence which is essential for initiating export. Confirmation of the location of the mutations in the portion of the *malE* gene corresponding to the signal sequence would provide direct evidence for its role in the initial steps of protein secretion.

Fusion strains have also been used to isolate mutants defective in the localisation of the *lamB* gene product, the phage λ receptor¹⁴. These mutations most probably result in alterations in the signal sequence of this protein which is normally located in the outer membrane of *E. coli*.

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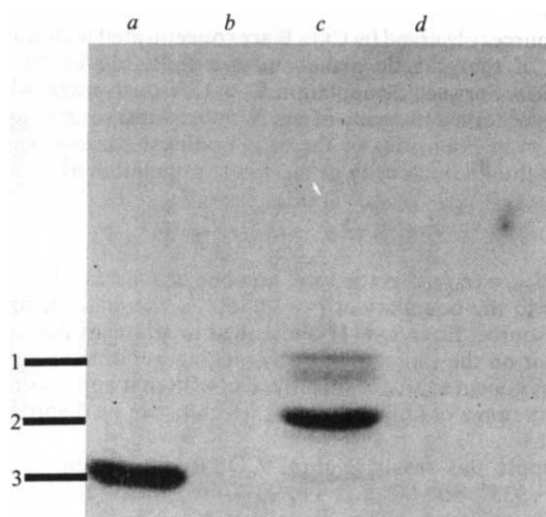


Fig. 5 Immune precipitation of MBP from soluble extracts of strains MC4100 and PB1101. 500 ml cultures were grown in maltose minimal medium to mid-log phase and labelled with ¹⁴C-amino acids (50 μ Ci, NEN) in a 50 ml volume for the final 60 min of culture. Cells were washed twice and resuspended in 5 ml of buffer (10 mM Tris, pH 7.2). Cells were then disrupted in a French pressure cell and twice clarified by centrifugation at 100,000 g for 1 h. 0.2 ml of the soluble cell extract, 0.1 ml [3 M NaCl, 3% Triton X-100, 10 mM Tris pH 7.2] and 3 μ l of rabbit anti-MBP serum (provided by W. Boos) were combined and incubated on ice for 3 h. 30 μ l of sheep anti-rabbit IgG (Miles) were added, and the entire mixture was incubated overnight at 4°C. The precipitate obtained was washed three times with 10 mM Tris, pH 8.0 (1st wash, 1% Triton X-100; 2nd wash, 0.1 M LiCl; 3rd wash, 0.1% SDS). The final pellet was resuspended in 0.1 ml gel sample buffer, heated at 90°C for 2 min, and 50 μ l were loaded per well for electrophoresis. Finally, the gel was stained with Coomassie blue, dried and autoradiographed. a, And b, strain MC4100; c, and d, strain PB1101; b, and d, anti-MBP serum was pre-incubated for 30 min on ice with 10 μ g of unlabelled native *E. coli* MBP. The three lines along the left side of the gel indicate the corresponding positions where the following three proteins are observed to run: (1), The MBP precursor observed in whole cells of strains PB1101 and PB1102. (2), The maltose-inducible protein observed in soluble cell extracts of strains PB1101 and PB1102. And (3), purified native *E. coli* MBP.

letters

The discrete source component of galactic γ rays

THE question of the relative contributions from discrete sources and continuum to the flux of cosmic γ rays is a continuing one, with published estimates varying from a 10% source contribution¹ to more than 40%² and even to a possible 100%³. Since these estimates have mainly been based on parts of the necessarily incomplete set of only 13 known γ -ray sources⁴, it is not surprising that such a wide variety of conclusions is possible. The basic reason is the limited angular resolution of contemporary γ -ray detectors ($\approx 1.5^\circ$), and it is unlikely that great improvements will be made in the near future.

A knowledge of the individual sources of γ rays (and their summed intensity contribution) is relevant to energetic processes in discrete objects, but our own prime interest relates to the fact that useful conclusions about the origin of the particle component of cosmic radiation can be obtained by an analysis of the continuum.⁵ The derivation of the magnitude of the continuum after both seen and unseen discrete source contributions have been subtracted off is, therefore, an important task.

Some general guidance might be expected from an analysis of related regions of the electromagnetic spectrum. In X rays, where detectors of high angular resolution are used, the continuum contribution appears to be very small (probably $\leq 5\%$); however, that the observed γ rays are not the high energy 'tails' of the X-ray sources is immediately clear from the general lack of correlation of specific γ -ray and X-ray sources.

A comparison with the radio region is probably more relevant. Potential candidates for producing the continuum γ rays are electrons of energy of the order of 1 GeV interacting with the interstellar medium by way of bremsstrahlung and protons of energy 1–10 GeV interacting with the same medium and producing π^0 mesons which in turn decay to γ rays. The relevance of the radio region is that it is generally accepted that the bulk of the radio flux (in the range 10–1,000 MHz) is produced as synchrotron radiation from electrons spiralling in the galactic magnetic field. One GeV electrons produce radiation with frequency 150 MHz and Brindle *et al.*⁶ have presented evidence which shows that the continuum radiation provides at least 95% of the flux. Thus, such indirect evidence as there is suggests a predominance of the continuum contribution.

It is apparent from an examination of the longitude distribution of the total γ -ray flux that sources distributed in a similar fashion to main sequence stars are not responsible for much of the flux. The expected large peak towards the galactic centre is absent. Furthermore, the latitude distribution of γ rays is much sharper than that of starlight. Instead, it is likely that the sources have a similar spatial distribution to that of young objects (such as, HII regions), evolved objects (pulsars, supernova remnant) or X-ray sources (the fact that there is not a one-to-one correspondence with known X-ray sources does not preclude this).

We aim here to derive more reliable estimates of the discrete source contribution using both a simple geometrical model and properties of X-ray sources and pulsars as a guide. Since these different approaches lead to very similar results there is some justification for confidence in their validity.

We consider separately the contributions from two possible types of source population: (1) sources with high intrinsic luminosity and low space density (HP sources) and, (2) sources with low intrinsic luminosity and high space density (LP sources).

In the simplest version of the model the sources are distributed uniformly throughout a slab of half thickness z_0 . The differential source count frequency from HP sources will then be $N(S) \propto S^{-2}$ for distances r much greater than z_0 and the sources will be closely confined to the galactic plane, while LP sources are numerous enough to give a large number nearer than $\approx z_0$, these nearby sources being isotropically distributed on the sky. This differentiation of source populations shows very clearly the dependence of the derived limits on the space density of the sources. The use of this double δ -function luminosity function is adequate provided we restrict attention to low latitudes where geometry should influence $N(S)$ more than the luminosity function.

The sources observed by COS B are concentrated within a few degrees of the galactic plane and are therefore of the HP population. For such a population $S_N \propto 1/N$ on average, where S_N is the observed intensity of the N 'th brightest source. Using the observed intensities of the N_{obs} brightest sources we can estimate the total intensity of the whole population as

$$J_T = \sum_1^{N_{\text{obs}}} S_N \cdot \sum_1^{N_{\text{max}}} \frac{1}{N} / \sum_1^{N_{\text{obs}}} \frac{1}{N} \quad (1)$$

where $N_{\text{max}} = (r_0/r_1)^2$ is the total number of sources within the distance to the boundary at $r = r_0$ and r_1 is the distance to the nearest source. Equation (1) is intended to minimise the statistical error on the estimate of J_T by making use of the summed flux from known sources explicitly. (As written it applies simply to a small range of longitudes over which r_0 can be regarded as constant.)

We apply this result to three COS B observation regions $l = 305^\circ$ – 335° , 60° – 90° and 110° – 140° for which a systematic search for sources has been made⁴ and $N_{\text{obs}} = 8$. The anticentre region has been excused since two-dimensional geometry is probably inappropriate there. Equation (1) then becomes

$$J_T = J_8 \times 0.37(0.62 + 2 \ln(r_0/r_1)) \quad (2)$$

From ref. 4, $J_8 = 3.8 \times \text{Crab flux} = 1.3 \times 10^{-5} \text{ cm}^{-2} \text{ s}^{-1}$ ($>100 \text{ MeV}$) (using the measured Crab flux of $3.5 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$). The main uncertainty is the value of r_0/r_1 , although this is not critical because of the logarithmic dependence in equation (2). Since the bright observed sources have latitudes $|b| = 0$ – 3° , the magnitude of r is in the range 1–2 kpc if the thickness $2z_0$ of the galactic source layer is about 200 pc. (a value typical of young objects). The value of r_0 lies in the range 10–20 kpc for the regions considered, so that $J_T = (1.4$ – $2.4)J_8 = (1.8$ – $3.1)10^{-5} \text{ cm}^{-2} \text{ s}^{-1}$ and the average line flux is $(0.9$ – $1.5)10^{-5} \text{ cm}^{-2} \text{ s}^{-1} \text{ rad}^{-1}$ ($>100 \text{ MeV}$). The average observed total line flux for these regions is $3.8 \times 10^{-5} \text{ cm}^{-2} \text{ s}^{-1} \text{ rad}^{-1}$ (for $|b| < 6^\circ$) so the analysis indicates that between 24% and 40% is from sources.

Source density gradients in the Galaxy may make the use of a uniform two-dimensional geometry inappropriate for the $l = 305$ – 335° region because one is here looking in a direction not far from that of the galactic centre; however, for the regions $l = 60^\circ$ – 90° and 110° – 140° , where the gradient is probably small, the average line flux is $2.8 \times 10^{-5} \text{ cm}^{-2} \text{ s}^{-1} \text{ rad}^{-1}$ ($|b| < 6^\circ$)

Table 1 Alternative estimates of the fraction, f , of γ rays above 100 MeV from discrete sources

Model	Longitude intervals (γ -ray observations)	N	J_T/J_N	$j_S \times 10^5$ $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ ($>100 \text{ MeV}$)	f
Flat slab	1, 2, 4	8	1.4–2.4	0.9–1.5	0.24–0.40
	1, 2	8	1.4–1.9	0.9–1.2	0.32–0.42
X-ray sources	1, 2, 3, 4	12	1.1	0.9	0.19
Pulsars	1, 2, 3, 4	12	1.05	0.9	0.18

Longitude key: 1, 60°–90°; 2, 110°–140°; 3, 160°–210°; 4, 305°–335°.

and $r_1 \approx 10 \text{ kpc}$. Hence $J_T = (1.4\text{--}1.9)J_8$ and the discrete source fraction is 32–42%.

A second approach to the HP component follows ref. 3 in assuming that the form of the observed brightness distribution $N(S)$ for X rays is similar to that for γ rays. Since the known sources do not have a constant ratio of γ -ray to X-ray flux, as remarked earlier, we postulate that while γ -ray sources are different objects from X-ray sources, their spatial distribution, luminosity function and total number are approximately the same as for X-ray sources. Since both populations have their brightest sources confined to within a few degrees of the plane a similar spatial distribution at least seems probable. In applying this method we again confine attention to the COS-B observation regions which have been systematically searched for γ -ray sources (the anticentre region can now be included since no particular geometry is assumed). The 72 4U sources⁷ in these four areas are found to have a value of J_T/J_N for X-rays which is smaller than for a simple slab model (a consequence of the flattening of the X-ray $N(>S)$ to $S^{-0.4}$ below 200 Uhuru counts). This method gives a slightly lower value for f than the slab model (Table 1). It is interesting to note that if the $N(>S)$ versus S distributions for X-ray and γ -ray sources are normalised at the S intensities for the Crab, then the value of f is virtually the same as found using the method described above.

Although the particular assumptions made in the $N(S)$ method are unlikely to be completely justified the comparison of this result with that from the slab model does indicate that the sensitivity of the estimates to the type of (HP) source population involved is small enough to make the exercise worthwhile.

Another useful deduction from the X-ray data can be made as follows. A prominent feature of the X-ray sources is that there is a much bigger intensity in the general direction of the Galactic Centre than elsewhere. Restricting attention to X rays (and γ rays) within a latitude range of $|b| < 10^\circ$ the ratio of total X-ray intensity in the inner Galaxy to that in the outer is $I_X(270^\circ < l < 90^\circ)/I_X(270^\circ > l > 90^\circ) \approx 9.4$. This ratio can be compared with that for γ rays above 100 MeV of 2.2. (If the contributions from the Crab (which dominates in the X-ray case) are taken out the ratio is as high as 34 for X rays and only 2.4 for γ rays.) An upper limit can be set as the fraction of the flux of γ rays in the outer direction coming from discrete sources by assuming that the whole of the flux from the inner direction is from such sources. Adopting a ratio of 9.4, which is itself a lower limit, we find $f \approx 0.25$, that is, in the range $270^\circ > l > 90^\circ$ no more than 25% of the γ -ray intensity is produced by X-ray sources or sources distributed in the Galaxy in a similar fashion to X-ray sources.

For the third estimate of the HP contribution, we have taken the radio fluxes from the Arecibo deep pulsar survey⁸, covering $|b| < 4^\circ$, $43^\circ < l < 58^\circ$. This was chosen for its completeness to low radio fluxes, although the region covered is much more restricted than the COS-B observation areas. A similar result to that for X rays is obtained (Table 1). Comparison can be made with our earlier estimate¹ for pulsars ($f = 0.05\text{--}0.10$). The fact that the new value is a factor of 2 higher is not surprising since the new result refers to sources in general with an observed intensity distribution similar to that of pulsars whereas the

earlier value related to pulsars alone (with a model in which the γ -ray luminosity was proportional to the radio luminosity).

Turning to the LP sources, we estimate the minimum density of such sources which would be required to produce a fraction f of the galactic plane emission without being visible as discrete sources above the current COS B threshold of $S_{\min} \approx 0.2 \times \text{Crab flux}$. A uniform slab model with thickness $2z_0$ and radial extent r_0 gives a minimum source density

$$\rho = \left\{ \frac{1}{2z_0} \cdot f \cdot \frac{j_{\text{obs}}}{S_{\min}} \cdot \frac{1}{[1 + \ln(b_0 r_0/z_0)]} \right\}^3 \left(\frac{\Omega}{3} \right)^3 \quad (3)$$

where Ω is the solid angle of the area of sky considered and j_{obs} is the observed total line-flux. Using $z_0 = 0.1 \text{ kpc}$, $b_0 = 10^\circ$, $r_0 = 10 \text{ kpc}$, $\Omega = 1 \text{ sr}$ for the COS B regions $l = 60^\circ\text{--}90^\circ$, $110^\circ\text{--}140^\circ$ and $305^\circ\text{--}335^\circ$ gives

$$\rho \approx 10^5 f^3 \text{ kpc}^{-3}$$

or a total number of LP sources in the Galaxy of the order of $10^7 f^3$. For $f = 1$ this is about three orders of magnitude lower than that of main sequence stars, but two orders of magnitude higher than that of likely sources such as pulsars⁹ and a possible population of weak X-ray sources¹⁰ (up to 10^5 sources with X-ray luminosity $10^{32} \text{ erg s}^{-1}$). Alternatively, we can state that a maximum of about $100^{-1/3}$, that is, 20%, of the galactic emission could originate in sources of this latter density.

In conclusion, Table 1 shows that the most likely value of f is ≈ 0.25 although it could be as high as 0.4 (and not inconsistent with the estimate of Bignami *et al.*²). Such a value would not invalidate the interpretation of γ -ray data in terms of a gradient of cosmic rays in the Galaxy, at least in the outer Galaxy⁵. A final remark is that the so-called discrete sources of γ -rays will include complexes of molecular clouds which provide target nuclei for cosmic rays; a derivation of their contribution must await more comprehensive surveys of such cloud complexes.

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Creation of magnetic monopoles in pulsars

THE symmetry of the free Maxwell equations under transformation of electric field into magnetic field led Dirac¹ to suggest the existence of magnetic monopoles. Many experimental searches for these particles have been made on different accelerators and using cosmic ray interactions; no magnetic monopoles having been detected, upper limits of the cross sections for the creation of monopoles have been obtained. The data of Ross *et al.*² are the best and can be fitted by:

$$\sigma_{pn} \leq 5 \times 10^{-44} m_g^3 \quad (1)$$

where σ_{pn} is the proton–nucleon cross-sections in cm^2 , and m_g

the monopole mass in $\text{GeV } c^{-2}$. It is believed^{3,4} that very energetic particles are created in pulsars. These particles can interact between themselves and/or be smashed onto the neutron star surface to produce pairs of magnetic monopoles and antimonopoles. These monopoles (if they exist or are effectively produced) would be accelerated in the pulsar magnetic field, one species towards the interstellar medium, the other towards the neutron star, producing a decrease in the magnetic field. Such a phenomenon can be related to observations, particularly to the discrepancy between the characteristic and the kinematical ages. We show here that these observations can be explained by a production of magnetic monopoles, inherent to the pulsars, with a cross-section lower than equation (1), (that is, without contraction with the negative results of the terrestrial searches). More specifically, we will consider the magnetic monopoles production on the neutron star surface, in the Ruderman and Sutherland model⁴. Other models (with and without gaps) will be considered elsewhere.

Ruderman and Sutherland⁴ argue that the creation of polar gaps should take place in pulsars. These polar gaps would be broken by electron pair creation and the subsequent avalanche development, with the potential across the gap

$$V = 1.6 \times 10^{12} B_{12}^{-1/7} T^{-1/7} \rho_6^{4/7} \quad (2)$$

where $\rho = 10^6 \rho_6$ is the curvature of the magnetic field lines at the neutron star surface in cm. The creation of magnetic monopoles is then possible due to the electrons accelerated by the potential of equation (2) across the gap and which crash on the neutron star crust.

The energy threshold E_{eth} for creation of monopoles with the mass m_g on the crust nucleons of mass m_n is

$$E_{\text{eth}} \approx 2m_g^2 m_n^{-1} \quad (3)$$

With the energy eV given by equation (2), the electrons have enough energy to create monopoles with $m_g \leq 30 \text{ GeV } c^{-2}$. Inside the neutron star crust, the electrons lose their energy due to bremsstrahlung and the number of nucleons seen by the electrons before they are slowed down below the threshold energy for monopole creation is

$$\kappa_e \approx \beta X_0 \approx 8.4 \times 10^{24} \beta \text{ nucleons cm}^{-2} \quad (4)$$

where X_0 is the radiation length and $\beta = \log(eV/E_{\text{eth}}) \approx 1$ practically independent upon the pulsar properties. The flux of the magnetic monopoles pairs produced in one polar gap is

$$j_g = \kappa_e \pi r_p^2 n \sigma_{\text{en}} \approx 6.3 \times 10^{54} B_{12} R_6^3 T^{-2} \sigma_{\text{en}} \text{ monopoles s}^{-1} \quad (5)$$

where σ_{en} is the cross-section for the monopole creation in the electron-nucleon interactions. The radius of polar gap r_p and the density of created e^+e^- pairs, n , are given in ref. 4.

Kalman and Ter Haar⁵ pointed out that the magnetic monopoles are accelerated by the pulsar magnetic field up to 10^{18} eV and interacting with neutron star crust should produce a cascade of 'secondary' monopoles. They estimate the number of secondary monopoles produced by a primary monopole to be

$$N^* = 6 \times 10^7 B_{12}^{1/4} \rho_6^{1/2} n_g^{-1/4} m_g^{-1} \quad (6)$$

where n_g is the magnetic monopole charge in units of $137 e$. Half of the monopoles which could be produced in this model are accelerated towards the neutron star surface and with the multiplicity this gives a decrease in the pulsar magnetic field at a rate

$$\frac{dB}{dt} = -\frac{j_g N^* 137 n_g e}{4 R^2} \quad (7)$$

It is commonly assumed that the slowing down of the pulsar results from the loss of rotational energy by magnetic dipole radiation. This allows us to estimate the neutron star magnetic field as a function of the period T and of its derivative $\dot{T}$

$$B \approx 8.3 \times 10^{18} I_{44}^{-1/2} R_6^{-3} (T\dot{T})^{1/2} G \quad (8)$$

where $I = 10^{44} I_{44}$ is the moment of inertia of the neutron star in g cm^2 and $R = 10^6 R_6$ is its radius in cm. Equation (7) can then be

integrated and the magnetic field expressed as a function of the period T :

$$B(T) = B_i \left\{ 1 - \frac{\sigma_{\text{en}}}{\sigma_{\text{crit}}} \log \left(\frac{T}{T_i} \right) \right\}^{4/7} \quad (9)$$

with

$$\sigma_{\text{crit}} = 6 \times 10^{-46} \frac{R_6^5 B_{12}^{7/4}}{I_{44} \rho_6^{1/2} n_g^{3/4}} \text{ cm}^2$$

where T_i and B_i are the period and the magnetic field at the creation of the gap ($\approx$ the creation of the pulsar).

There are observations to test this decrease in the magnetic field. First, it implies that the characteristic ages $\tau = T/2\dot{T}$ are not true indicators of the real ages but must be greater. Actually there is a discrepancy between the kinematical ages τ_k (obtained from the direct measurements of the proper motion and the distance from the galactic plane of the pulsars) and the characteristic ages, which seem greater⁷. For those pulsars whose proper motion was measured, the mean kinematical age is $\langle \tau_k \rangle = 1.3 \times 10^6 \text{ yr}$ while the mean characteristic age is $\langle \tau \rangle = 5 \times 10^6 \text{ yr}$, and if the same mean velocity is used for all the pulsars a much larger discrepancy is found. Furthermore, some pulsars are remarkable because of their extraordinarily great characteristic ages—up to 10^9 yr —a fact which could very well be explained by a decrease in the magnetic field, too. Considering equation (9), it can be seen that the creation of magnetic monopoles in pulsars may explain these observations if $\sigma_{\text{en}} \leq \sigma_{\text{crit}}$. When this cross-section is compared with the upper limit of equation (1) from the monopole searches using cosmic rays, it can be seen that there is no contradiction with this explanation and the fact that monopoles are not observed directly: expecting that the alpha-numeric coefficient $R_6^5 B_{12}^{7/4} / I_{44} \rho_6^{1/2}$ to be of the order of unity, we have for the monopole production cross-section in pulsars:

$$\sigma_{\text{en}} \leq 6 \times 10^{-46} \frac{m_g}{n_g^{3/4}} = 3 \times 10^{-44} \text{ cm}^2 \quad \text{for } m_g = 30 \text{ GeV } c^{-2} \text{ and } n_g = 0.5 \quad (10)$$

which is considerably smaller than the terrestrial upper limit of equation (1):

$$\sigma_{\text{pn}} \leq 10^{-39} \text{ cm}^2 \quad \text{for } m_g = 30 \text{ GeV } c^{-2} \quad (11)$$

(Besides, the interactions for the production of monopoles in pulsars involve high energy electrons, while the upper limits published so far concern nucleons.) Therefore, as an explanation of the discrepancy between τ and τ_k and of the very great characteristic ages, we suggest that this comes from a decrease in magnetic field due to monopole creation in pulsars with the cross-section of equation (10). This provides an indication of the existence of the magnetic monopoles and of their creation in pulsars or at least (if another explanation for the observations is preferred) a new upper limit on the monopole production cross-section, far more stringent than the terrestrial ones.

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Possible nuclear origin of Ap and Am stars

MANY possible mechanisms have been proposed to explain the abundance anomaly of heavy elements on Ap and Am stars. Van den Heuvel¹ suggested that these stars had been secondaries of binary systems, whose primaries exploded as supernovae and caused the accretion of heavy elements on the secondaries. The abundance distribution of elements with $Z \geq 40$ in those stars is generally similar to that of r nuclei in the Solar System, although the overabundance factor for each element relative to Solar System r abundance is not constant, being exceptionally high in some limited regions such as the region around Te, the rare earth region around Nd and Sm, and around Pt and Hg. These observational trends suggest that the abundance pattern in those stars may be a superposition of some specific elements with anomalously high abundance on a background proportional to the Solar System r abundance. Here I suggest an alternative possibility for the nuclear origin of anomalous abundance in Ap and Am stars.

In the early expanding period of supernova shells, the Rayleigh–Taylor instability², plasma instabilities or, at a later period, some instability accompanying dust formation³ would occur. These instabilities might cause more or less pronounced density and composition inhomogeneities and chemically inhomogeneous clumps or knots of clouds might appear in the expanding shells. The clumps would have envelopes surrounding them², so that the movement of these clumps would generate turbulence in the expanding shells and tend to chemically homogenise the supernova shells. We can therefore imagine that in the expanding supernova shells there are chemically inhomogeneous clumps embedded in and co-expanding with the chemically homogeneous background gas. These clumps would appear $\leq 4 \times 10^{14}$ cm from the supernova². Taking the distance between the components of a typical binary system to be

$\sim 10^2$ AU, the instabilities in the shells would occur during the transfer towards the secondary, and the accreting matter on the secondary would probably be composed of some clumps and the background gas, now partially chemically homogenised.

If we restrict the discussion to the region $Z \geq 40$, the nuclei formed in the supernova shells are only r and p nuclei. Using Arnett's model⁴ of the presupernova with $M_a = 8M_\odot$, I have investigated the nuclear synthesis occurring in the innermost shell of exploding supernovae⁵. The r process is found to take place when the star is within the lagrangian mass range $M_r = 1.34\text{--}1.69 \times 10^{33}$ g. The number of free neutrons per seed nucleus gradually increases with the decrease of M_r in that lagrangian mass range, and therefore the appearance of the abundance curve of produced r nuclei varies with position in the r shell. The shell-integrated abundance over the r shell, however, can well reproduce the relative abundances of r nuclides in the Solar System. If the r nuclei are produced in the above lagrangian mass range, they become overabundant in the supernova shells as a whole by a factor of $\sim 3 \times 10^4$ as compared to the Solar System r abundance, whereas the overproduction factor for the p nuclei is 10^{0-3} only in the p nuclei-producing region⁶. Hence in the region $Z \geq 40$ the abundance distribution in the background gas which accretes on the secondary star would be substantially that of the r process. However, some accreting clumps with a high abundance of r elements might be produced at some position in the r shell. Where these clumps accrete, chemically inhomogeneous spots with intrinsic distribution of r elements would form. It would therefore be possible to observe the abundance distribution due to these spots, being superimposed on the background distribution. The extent of the strong overabundance in some specific elemental regions depends on the mass of accreting clumps relative to the mass of accreting homogeneous gas.

Cowley⁷ points out that in the rare earth region the abundance of individual elements in Ap and Am stars can be expressed as two components—the ordinary abundance pattern with a normal odd-even effect (component A), and an abundance distribution with a maximum centred around intermediate rare earths (component B). According to my proposed model, these components A and B correspond, respectively, to the background with the r abundance appearance and to the intrinsic component originating from a chemically inhomogeneous spot.

As an example, consider HR465 observed in 1960 at its rare earth maximum. Figure 1 shows the observations⁸⁻¹⁰ in the region of $Z \geq 40$ together with the theoretical abundances which consist of the 'background' (this is proportional to the r abundance observed in the Solar System^{5,11}; component A) and two components B₁ and B₂ intrinsic for HR465. The components B_{1,2} have the r abundances produced in the regions around $M_r = 1.48$ and 1.37×10^{33} g, respectively. The calculated abundances have been obtained by the superposition of three components so as to minimise the deviation from observations. The relative numbers of nuclei with $Z \geq 36$ are

$$A : B_1 : B_2 = 1.0 : 35.5 : 0.45$$

The calculation can well explain the observed data within the observational uncertainties. The component B₁ makes the regions around $Z = 42, 52$ and 60 overabundant, while component B₂ has a maximum centred around Pt. The abundances observed in the other Ap and Am stars are also explained by superimposing some intrinsic abundance patterns on the 'background'. We also expect to observe stars which do not require an additional intrinsic component, and which are explained by the 'background' component. Stars such as 32 Aqr, 63 Tau and γ Equ = HD201601 might be of this type.

Figure 2 shows the mass fraction of the region where a given element is produced in excess to the total mass of the r shell. A given element is said to be in excess at some position i in the r shell when the quantity $N(Z) - \langle N(Z) \rangle$ becomes positive, where $N(Z)$ is the number of nuclei with Z per unit mass produced at i and $\langle N(Z) \rangle$ is the average value over the entire r shell. Figure 2 implies that, if the sites for Rayleigh–Taylor

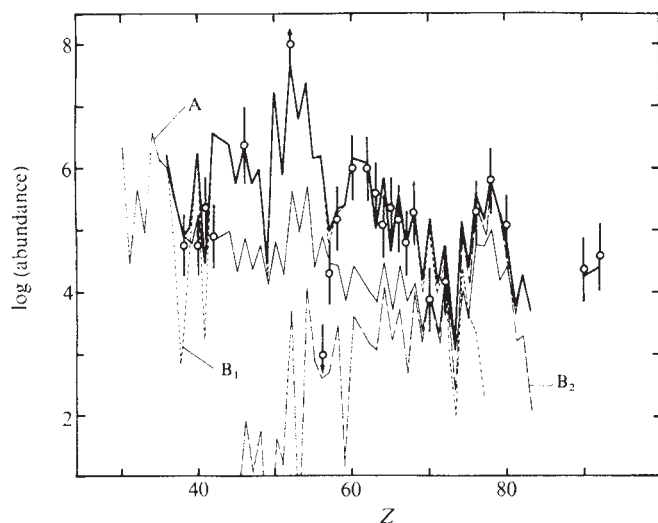


Fig. 1 Abundances of heavy elements on HR465 in the scale $H = 10^{12}$. Open circles with error bars show observations, whereas the heavy solid lines denote the calculated abundances. Arrows indicate the data with only upper or lower limits. Observational uncertainties are unclear but are assumed here arbitrarily to be $\pm \text{dex}(0.5)$, although they could be higher. The calculated abundances are composed of the background (A) and two components B₁ and B₂ intrinsic for HR465. They are shown by light solid, short dashed and long dashed lines, respectively. Abundance patterns for the component A in the region of $Z \geq 90$, B₁ in $42 \leq Z \leq 68$ and B₂ in $74 \leq Z \leq 80$ are substantially the same as those given by the heavy solid lines.

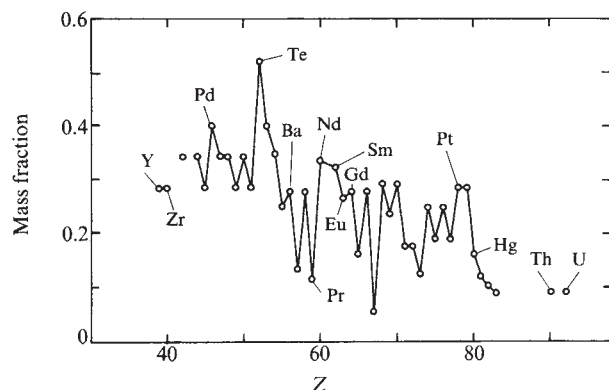


Fig. 2 Fraction of the mass of the region where a given element is produced in excess relative to the total mass of the r shell. Atomic symbols are given for representative elements frequently observed in Ap and Am stars.

instability or plasma instability are randomly distributed in the r shell, the probability that elements near $Z = 52$ will appear in Ap and Am stars with exceptionally high overabundance factors compared to the other elements is by about twofold higher than the probabilities for the other elements. Furthermore, in the light rare earth region the probability is slightly higher than that for heavy rare earths. The occurrence of Ap and Am stars with a high overabundance factor for a given element would show a pattern similar to that in Fig. 2 provided my hypothesis is the only mechanism operative.

Although the hypothesis well accounts for the gross feature of abundance curves on the Ap and Am stars, it cannot always explain the details of the curves; for example, it fails to explain the low abundances of Ba, La and Yb in HR465. For a complete and detailed explanation, secondary processes such as the magnetic accretion or the diffusion process would have to be introduced.

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Consequences of the day-to-night variation of the exospheric temperature of Venus

DURING the exploration of the planet Venus by the Soviet space probe Venera 9, we measured the quantity of hydrogen in the upper atmosphere and its temperature through the optical observation of far UV atmospheric emission. The temperature at the altitude of 250 km (the upper boundary of the atmosphere) was found to vary from 500 K on the dayside to 200 K on the nightside. This variation is compatible with our current knowledge of the heating of upper atmosphere by the solar radiation. It is shown here that this variation, which is larger than on Earth, induces an exospheric transport (out of the atmosphere) of hydrogen from the dayside to the nightside. As an

indirect consequence, this transport allows the existence of a large quantity of atomic oxygen in the upper atmosphere, a feature which was still controversial.

Atomic hydrogen in the upper atmospheres of planets is a good tracer of the exospheric temperature¹. As it is produced through photochemistry of hydrogen compounds in the chemosphere, its distribution and total quantity are connected mainly to the mixing ratio of hydrogen compounds and to the efficiency of vertical transport^{2,3}. As hydrogen atoms are scattering Ly α solar photons, they can be easily observed optically, from outside the atmosphere of the planet, through their Ly α emission at 1,215.6 Å.

The Ly α photometer which was flown in October 1975 in the orbiter part of Venera-9 around Venus, allowed intensity and line shape measurements to be made, which have been described previously⁴. The detector was a NO counter used in a photon counting mode, measuring the Ly α emission I_0 through a hydrogen absorption cell in a field of view of 20'. When the cell is activated at an optical thickness $\tau = 10 \pm 1$ (with the help of a tungsten filament which dissociates H₂ molecules into atoms), a part of the Ly α emission line is absorbed in the cell, and the intensity measured by the NO counter drops to $I_r = RI_0$. The reduction factor R is a function of the emission line profile and of the Doppler shift of the spacecraft along the line of sight⁵.

Our previous report⁴ was devoted to the analysis of the data obtained on the sunlit part of the atmosphere above the planetary disk. A hot component was found to be present about 3,000 km of altitude, and associated with the possible effect of the solar wind on the hydrogen exosphere, when it becomes turbulent after bow shock crossing. Below 3,000 km of altitude, the exospheric temperature T_e was found to vary on the dayside from one day to the other between 400 and 600 K, with a mean value of 500 K, associated with a density of 1.5×10^4 atom cm⁻³ at 250 km, the exobase level.

This report analyses the measurements of intensity and reduction factor obtained for lines of sight intersecting the planetary disk, both in the dark and bright parts. These measurements are compared with model predictions of intensity and linewidth, built from a vertical distribution of hydrogen and

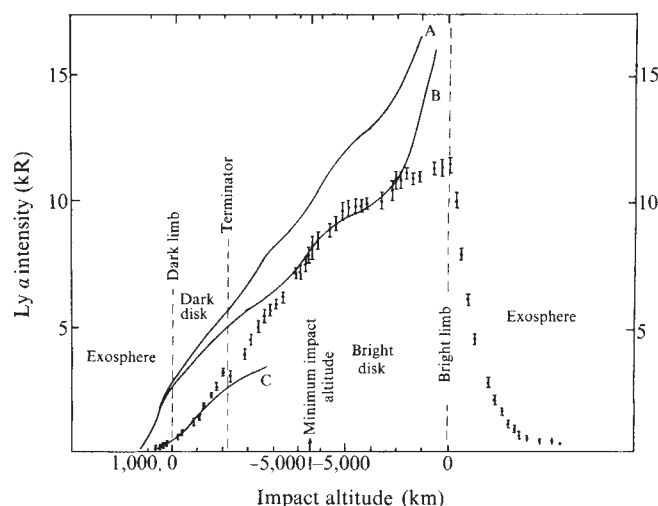


Fig. 1 The reduction factor (which measures the effect of the absorption cell on the Ly α signal when it is activated) is plotted as a function of impact altitude of the line of sight, counted negatively when the line of sight intersects the planetary disk. Theoretical curves are shown for various exospheric temperatures for comparison. The general decrease from left to right (increase of cell absorption) is due to decreasing Doppler shift. On the bright disk, the difference between measured and computed is attributed to contamination by O(I) 1,304 Å line. The observed temperature increase in the exosphere is attributed to bow shock interaction of decelerated solar wind protons with the hydrogen corona⁴.

the solution of the radiative transfer equation in the approximation of a spherically symmetric exosphere⁶. The line profile is a by-product of the radiative transfer solution⁷; a theoretical value of the reduction factor R can then be computed, as the Doppler shift is known.

There is some contribution of the large emission of O(I) at 1,304 Å to our measured intensity signal over the bright disk data, as in the case of the Earth, even if our NO counter is less sensitive by a factor of ≈ 3 at 1,304 Å than at Ly α . We have used the hydrogen cell measurements for estimating the stray contribution of O(I) as follows.

The values of R measured over the disk are compared to the theoretical values computed for three hydrogen vertical distribution: same vertical distribution below 250 km with density at 250 km $n_c = 1.5 \times 10^4$ atom cm⁻³, but three different exospheric temperature $T_c = 400, 600, 800$ K (Fig. 1). All curves, experimental and theoretical, decrease from left to right when the line of sight of the instrument scans the planet because during the same time, the Doppler shift due to the orbital motion decreases from -7.7 to -2.6 km s⁻¹ and therefore the absorption peak in the cell moves towards the centre of the Venus emission line. When the Doppler shift is equal to the half-width of the absorption profile, one half of the emission line is absorbed and the other is not, whatever the line profile (within limits relevant to the present discussion), and therefore at that point all theoretical curves intersect at a value of $R = 0.5$ (ref. 8). The fact that, for this particular value, the measured R is > 0.5 indicates the presence of a non absorbed signal which can be identified with the O(I) contribution to our signal and provides an estimate of its value. On all six orbits analysed, a subtraction of 23% of the bright disk intensities does bring back the measured values of R among the theoretical curves; this corresponds to a 0.8 ratio of the intensities $I(O(I))/I(H)$, in fair agreement with previous measurements⁹.

After subtraction of O(I) contamination (on the bright disk only), the intensity measurements were compared to theoretical intensities (Fig. 2) corresponding to two distributions of hydrogen. Both have the same exospheric distribution determined by $n_c = 1.5 \times 10^4$ at cm⁻³ at 250 km and $T_c = 500$ K (parameter deduced for the daylight exosphere from our previous analysis⁴). Below 250 km, the density follows the Liu and Donahue¹⁰ model distribution with the following cloud top volume mixing ratios: $f_{HCl} = 6 \times 10^{-7}$, $f_{H_2} = 7 \times 10^{-8}$, $f_{H_2O} = 3 \times 10^{-6}$ for model B and $f_{H_2O} = 2.5 \times 10^{-6}$ for model A. Both models have the eddy coefficient K taken at 10^8 cm² s⁻¹ and the limit of complete absorption of Ly α by CO₂ is set at 110 km. Differences between numerical values used by Liu and Donahue and us are of minor importance: we took, as our measurements indicated we should, $T_c = 500$ K instead of 350 K, and $n_c = 1.5 \times 10^4$ cm⁻³ at 250 km instead of $n_c = 10^5$ at 210 km. This last point corresponds to scaling down the Liu and Donahue density distribution by a factor of 5. According to the analysis of Sze and McElroy¹¹, this should imply approximately a decrease of hydrogen compounds at the cloud top level by the same factor of 5.

The results of the comparison are: (1) On the dark side, the theoretical intensities are nearly insensitive to hydrogen content below 250 km, region not illuminated: the main contribution comes from the part of the line of sight directly illuminated. Owing to the orbital configuration, this region of emission covers the twilight region behind the terminator, from 90° to roughly 120° of solar zenith angle. The measured intensities are much smaller than on either model, indicating that on the nightside of Venus the exosphere contains much less hydrogen than on the dayside. As shown on Fig. 2, a good fit is obtained with model C, identical to B below 250 km but with a lower temperature $T = 200$ K. Even lower temperatures would be required, if a density at exobase level was taken larger on the nightside than on the dayside, by analogy with the case of the Earth where the night-to-day ratio of hydrogen densities at the exobase level is of the order of 2, with a day-to-night temperature ratio of 1.3. We conclude that a large difference of exosphere temperature between day and night is demonstrated on

Venus. A similar conclusion was reached from Mariner 5 results¹², but with a more complicated analysis restricted to limb measurements. Such a large day-to-night temperature ratio is indeed predicted from an elaborate theoretical model¹³ of the upper atmosphere of Venus, which includes as a parameter the degree of efficiency of solar heating. Our dayside temperature of 500 K would correspond in this model to a medium efficiency solar heating.

(2) On the bright disk, the intensity is very sensitive to the hydrogen content below 250 km. A good fit is obtained with model B except near the bright limb, where field of view effects may smooth out the large theoretical increase. A Liu and Donahue model scaled down to our values gives a satisfactory description of the situation. We therefore have to discuss their conclusions. With their mixing ratio of hydrogenous compounds at cloud top (total hydrogen mixing ratio at least 10^{-6}) the production of hydrogen is large and because there are only three sinks (Jeans escape, non thermal escape, downward transport and recombination described by the eddy coefficient K) and as the Jeans escape is small, either the non thermal escape flux ϕ_{nt} or the eddy coefficient K must be very large. This places constraints over oxygen. If K is large, oxygen atoms are removed by transport and the dayglow at 1,304 Å is not due to optical resonance but to electron excitation. If K is small, oxygen atoms exist in large quantities and the dayglow is due to optical resonance, but in this case a very large non thermal escape flux is needed. The modifications our data force on the Liu and Donahue analysis ($T_c = 500$ K instead of 350 K, n_{250} scaled down by a factor of 5 which implies a value of f_{H_2O} around 0.5×10^{-6} at cloud top) do not change their conclusion. But what may change the discussion is the large asymmetry between day and night that we have demonstrated. On Earth, the density ratio adjusts itself to the temperature ratio in such a way that, at each point of the exobase, the upward flux of ballistic hydrogen atoms (a function of local conditions) is approximately equal to the downward flux of ballistic hydrogen atoms which comes from everywhere on the exobase. This seems not to be the case on Venus: the large difference in temperature between day and night, which results probably from the slow rotation of the planet, implies a large transport of hydrogen from the dayside to the nightside (or lateral flow) and the net upward ballistic flux

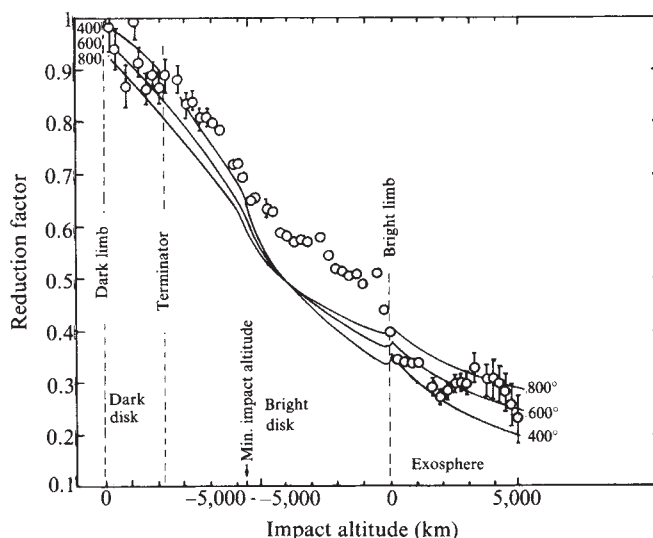


Fig. 2 The measured Ly α intensity (after subtraction of 1,304 Å contribution on the bright disk) is compared with computed intensities, for three different models A, B, C of hydrogen vertical distribution. A and B are different only below the exobase ($T_c = 500$ K) which shows that bright disk intensity is sensitive to hydrogen below the exobase. B and C are different only above the exobase, where model C is at $T_c = 200$ K. Dark disk intensity is sensitive to the hydrogen above the exosphere, directly illuminated by the Sun. $n_c = 1.5 \times 10^4$ atom cm⁻³.

can become positive. For a sinusoidal variation of the temperature between $T_{\max} = 500$ K at the subsolar point and $T_{\min} = 200$ K at the opposite point, and a uniform density distribution $n_e = 1.5 \times 10^4$, we computed this net upward ballistic flux to be 5×10^7 atom $\text{cm}^{-2} \text{s}^{-1}$ for the subsolar point. This upwards flux could very well play the role of the nonthermal escape flux ϕ_{nt} introduced by Liu and Donahue¹⁰ and Sze and McElroy¹¹. When reported on their Figs 2, 3, 4, $\phi_{nt} = 5 \times 10^7 \text{ cm}^{-2} \text{s}^{-1}$ implies low values of K of the order of $10^6 \text{ cm}^2 \text{s}^{-1}$. This value could, therefore, allow the atomic oxygen to build up and the 1,304 Å emission would be explained by resonance scattering.

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Direct experimental verification of the Kelvin equation for capillary condensation

THE thermodynamic properties of liquids trapped in microscopic pores or existing as very small, highly curved droplets are described by the Kelvin equation¹. This equation forms the basis of critical nucleation theory² and has been used in interpreting such diverse phenomena as adhesion³, the enhanced solubility of small particles² and the retention and flow of liquids in porous materials⁴⁻⁶. The validity of the application of the Kelvin equation to such highly curved interfaces (where the mean radius of curvature can be in the range 1-100 nm) has been questioned^{1,4}, but has never been tested by direct experiment. We have used multiple beam interferometry to observe the formation of capillary condensed liquid between crossed cylinders of molecularly smooth mica. We report here that the Kelvin equation is obeyed by cyclohexane menisci with mean radius of curvature as low as 4 nm. We further conclude that the application of the laws of thermodynamics, and the concept of a bulk surface tension, are valid in principle for such highly curved interfaces.

The Kelvin equation¹ (equation (1)) gives the equilibrium mean radius of curvature r_m of a liquid-vapour interface in terms of the actual vapour pressure p and the saturated vapour pressure p_s . The equation can be applied to both liquid drops (r_m positive) and to bubbles and capillary-held wetting liquids (r_m negative)². We have measured the mean radius of curvature of cyclohexane menisci at the periphery of liquid bridges trapped between two crossed cylindrical mica surfaces (Fig. 1) at equilibrium with relative vapour pressures in the range 0.71 <

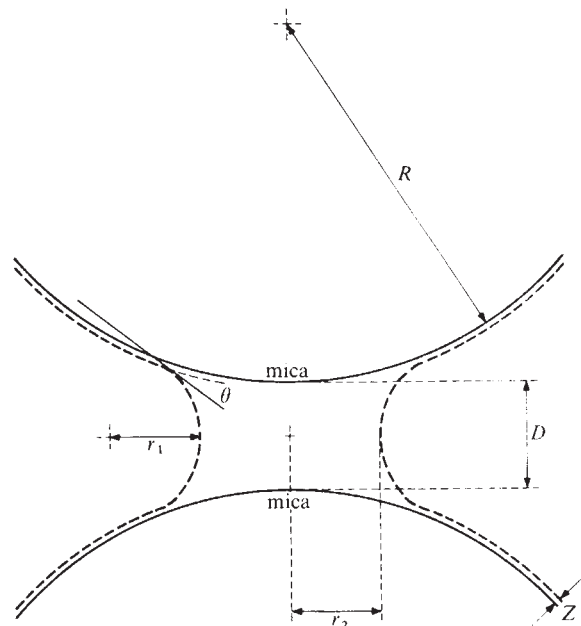


Fig. 1 Space between crossed mica cylinders with bridging meniscus (section through 45° plane of symmetry). Not drawn to scale: $R \approx 10^7$ nm, $r_2 \approx 10^4$ nm, $r_1 \approx -10$ nm. The mean radius of curvature r_m is defined as $1/r_m = 1/r_1 + 1/r_2$. By convention, r_1 is negative and r_2 positive in the configuration shown here. The equation used to calculate values of r_m from experimental values of the critical distance $D = D_c$ (see text) was $r_m = r_1 = -(D_c - 2Z)/2 \cos \theta$. This equation is accurate to within 0.5% for the range of values of r_m examined. The measured value of the contact angle θ was $\theta = (6 \pm 1)^\circ$ (L.R. F., to be published).

$(p/p_s) < 0.94$, giving mean radii of curvature in the range 4 nm < $-r_m$ < 19 nm. The experiments were carried out using an apparatus⁷ in which the distance D between the molecularly smooth surfaces of a pair of rigidly mounted crossed cylindrical mica sheets was controlled and measured to ± 0.1 nm. Distances were measured by multiple beam interferometry, which also

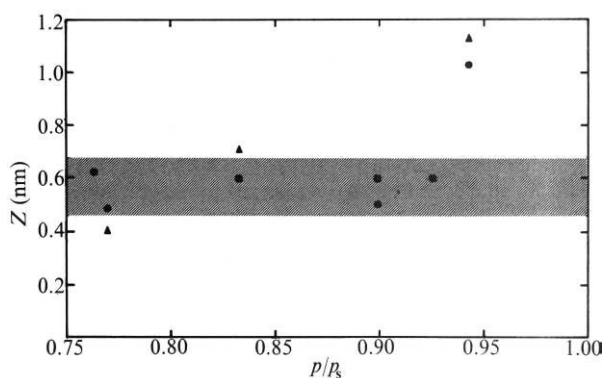


Fig. 2 Adsorption isotherm of cyclohexane on cleaved mica. Values of the adsorbed film thickness Z were determined (1) by measuring the distance (D_{contact}) between the mica surfaces at the moment of first contact, and assuming that $Z = D_{\text{contact}}/2$; or (2) by calculation from the measured root mean square refractive index (μ_{rms}) of the medium between the surfaces when the surfaces were held at a constant small distance (D) apart⁸. Δ , Points calculated from spacing at contact; $\circ$, points calculated from r.m.s. refractive index. Shaded band: range of possible values of the thickness of a cyclohexane monolayer, calculated from measurements on a Courtauld spacefilling molecular model. We have taken $Z = (0.6 \pm 0.1)$ nm for relative pressures below 0.93 and $Z = (1.05 \pm 0.1)$ nm for $p/p_s = 0.943$.

permitted observation of the presence of a bridging meniscus and measurement of the refractive index of the material between the surfaces⁸. For distances D greater than a critical distance D_c , the liquid bridge evaporated irreversibly. Values of r_m were calculated from the experimental values of D_c with allowance by simple subtraction for the thickness Z of the adsorbed film. Values of Z were determined from the adsorption isotherm at 293 K (Fig. 2).

The Kelvin relation between r_m and (p/p_s) is⁹:

$$\ln(p/p_s) = \{M/\rho_l RT\} \{(\gamma/r_m) - (p - p_s)\} \quad (1)$$

where M is the molecular weight, ρ_l is the liquid density, p/p_s is the relative vapour pressure, γ is the liquid-vapour interfacial tension at the absolute temperature T , and R is the gas constant. As $|\gamma/r_m| \gg |(p - p_s)|$ for the range of values of r_m measured, this equation gives an almost linear plot of $\ln(p/p_s)$ versus $1/r_m$, with a slope of $[\gamma M/\rho_l RT]$. Two theoretical plots of $\ln(p/p_s)$ versus

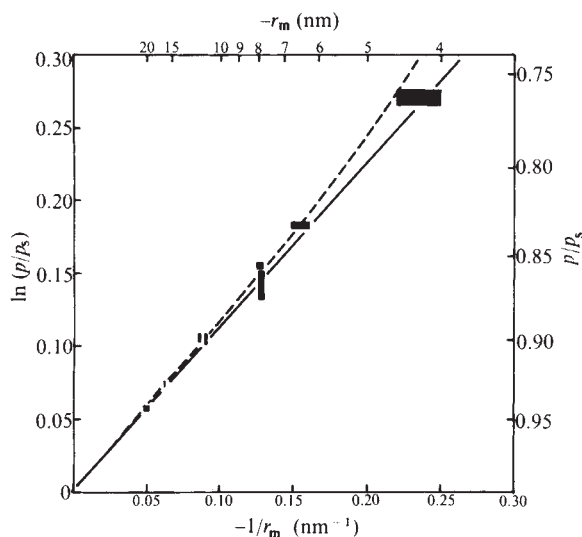


Fig. 3 Comparison of theoretical (Kelvin equation) plot of $-\ln(p/p_s)$ versus $-1/r_m$ with experimental results. The solid line corresponds to the assumption that γ ($=25.5 \text{ mN m}^{-1}$) is independent of r_m . The dashed line is calculated assuming the approximate relation¹⁰ $\gamma = \gamma_\infty(1 + \Delta\lambda/r_m)$, where γ_∞ is the surface tension of a planar cyclohexane liquid-vapour interface and $\Delta\lambda$ is the 'thickness' of the interfacial region. We have taken $\Delta\lambda = 0.49 \text{ nm}$ (ref. 12). The relative pressure p/p_s was controlled by maintaining a constant temperature difference between the mica surfaces and a pool of liquid cyclohexane in the chamber of the apparatus. Errors in (p/p_s) are due to temperature drifts during an experiment. The principal errors in r_m arise from uncertainty in the thickness of the adsorbed film (about $\pm 0.1 \text{ nm}$) and from errors in establishing the limits at which menisci were clearly observed to evaporate irreversibly (upper limit for D_c) or to grow (lower limit for D_c).

$1/r_m$ are shown in Fig. 3, together with the values of $1/r_m$ calculated from the experimental values of Z , D_c and θ at various experimental values of $\ln(p/p_s)$. The experimental points agree closely with those predicted by the Kelvin equation, assuming $\gamma = \text{constant}$, down to the smallest mean radius of curvature examined ($r_m = -4.2 \text{ nm}$). If allowance is made for the effect of curvature on surface tension¹⁰, a theoretical line is obtained with a slightly better fit to the experimental points. The assumption that an equilibrium adsorbed monolayer affects only the geometry of the system and not the meniscus curvature seems to be justified, although this assumption may need modification for thicker adsorbed layers¹¹.

In conclusion, we have verified that the Kelvin equation for cyclohexane condensed on to mica surfaces is satisfied to within

$\pm 6\%$ of r_m down to meniscus radii of 4 nm . A more detailed account of this work will be published elsewhere.

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Measurement of carbon tetrafluoride in the atmosphere

ATMOSPHERIC trace concentrations of carbon tetrafluoride (CF_4) in a variety of ambient air samples have been measured. The air samples were concentrated by a cryogenic trapping technique and the CF_4 species identified by combined gas chromatography-mass spectrometry (GC-MS) with single ion monitoring. The contribution of natural and/or anthropogenic sources to the global burden of CF_4 is unknown. CF_4 may be one of the atmosphere's rare gases.

CF_4 is a very stable compound that behaves like an inert gas up to a temperature of about $2,000^\circ\text{C}$. The presence of CF_4 in the atmosphere at a concentration of approximately 0.1 to parts per 10^9 (p.p.b.) was deduced by Gassman¹ from MS analyses of contaminant levels of CF_4 in high-purity krypton samples. Gassman suggested¹ that CF_4 was enriched from the atmosphere during the cryogenic preparation of high-purity krypton. The GC-MS analyses made at Harwell were conducted to determine whether CF_4 is indeed a common constituent of the ambient atmosphere, and if so at what concentration.

A pre-concentration technique was used to provide samples for GC-MS analysis. A glass U-tube equipped with Teflon stopcocks was immersed in liquid nitrogen and air was allowed to liquify until the tube was approximately two-thirds full. Clean helium was then bubbled slowly through the trap until the liquid air had evaporated. The trap was then removed from the liquid nitrogen and thermally equilibrated at room temperature. This created a positive pressure of helium in the trap containing enriched concentrations of the trace species present in the air at the time of trapping. Samples of the mixture were then simply taken off the side arms of the trap with a syringe and injected directly into the chromatographic column. The helium gas used in the enrichment process was cleaned before passage into liquid air by passing through 40 ft of stainless steel tube ($\frac{1}{8}$ inch outer diameter), immersed in liquid nitrogen. This effectively removed all CF_4 and Xe that may have collected in the liquid air trap in the evaporation process. Analysis of blanks made by passing helium through the cold empty trap for 30 min showed no signs of either CF_4 or Xe.

For quantification both ^{84}Kr (boiling point -158°C) and ^{132}Xe (boiling point -107°C) were measured directly in 5 ml air samples and in the cryogenic preparations. Essentially 100%

enrichments of ^{84}Kr and ^{132}Xe were obtained. Accordingly the high efficiency of the cryotrap for ^{84}Kr and ^{132}Xe bracketing CF_4 with its intermediate boiling point of -128°C was taken to indicate that CF_4 was quantitatively enriched. Subsequent analyses measured only ^{132}Xe for use as an internal standard in the CF_4 analyses. The concentration of ^{132}Xe in the air is 23 p.p.b. determined from its isotope abundance of $\sim 27\%$ of a total xenon atmospheric concentration of 84 p.p.b. Accordingly its constant atmospheric concentration provided an excellent internal standard for determining the enrichment ratios of the samples studied.

Analyses were performed on a Hewlett-Packard 5700 series gas chromatograph fitted with a $\frac{1}{4}$ inch o.d., 3 ft glass column packed with 70–140 mesh Woelm alumina GSC-120. Column temperature was held at -20°C to enrich the 10 ml injections of the cryotrap contents onto the head of the column. After elution of the air peak, at 2 min, the temperature was raised ballistically ($>32^\circ\text{C min}^{-1}$) to $+50^\circ\text{C}$. Helium purified by passage through liquid N_2 was used as the carrier gas to 40 ml min^{-1} .

The gas chromatograph was interfaced to a V.G. Micromass-16 mass spectrometer by a jet separator. Mass 69 (CF_3) was monitored as the major peak for the parent CF_4 species with a retention time of 3.2 min. ^{132}Xe was measured at 4.2 min.

A representative analysis of CF_4 and ^{132}Xe chromatographed from an enriched air sample from South Pole, Antarctica, is shown in Fig. 1. The peak heights of each compound were used for quantitation against a CF_4 calibration standard in air and the known atmospheric concentration of ^{132}Xe . The ^{132}Xe peak was used as an internal standard to determine the enrichment ratio obtained in processing the cryotrap. The gains of the output attenuation of the strip chart record are CF_4 , $10^{-8} \times 10$ and ^{132}Xe , $10^{-5} \times 5\text{ A V}^{-1}$.

The concentration of CF_4 was determined in 21 analyses of 11 different ambient air samples representative of the environs

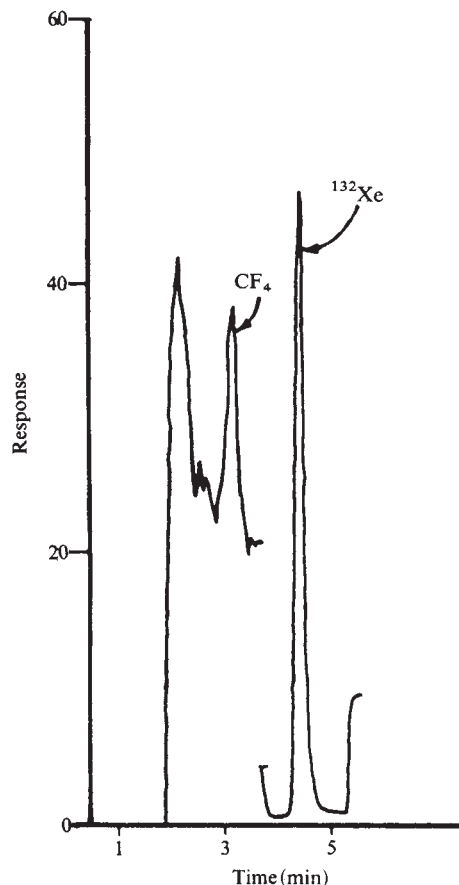


Fig. 1 GC-MS analysis of CF_4 and ^{132}Xe in air sample from South Pole, Antarctica.

Table 1 CF_4 Measurements

Date of analysis	Sample	Concentration factor	CF_4 (%) [*]
Harwell			
17/7/78	OA	30.8	56.5
17/7/78	OA	16.1	66
		20.3 repeat	42
17/7/78	OA	15.9	58
		16.3 repeat	55
17/7/78	OA	19.5	59
18/7/78	OA	30.95	55
		34.52 repeat	43
18/7/78	OA	25.97	83
18/7/78	OA	29.41	71
		116.18 repeat	85
18/7/78	OA	10.3	74
19/7/78	OA	36.62	83
19/7/78	OA	6.02	69
		5.26 repeat	56
19/7/78	OA	30.16	62
Other			
19/7/78	PNW 171	8.20	63
		37.50 duplicate	67
20/7/78	S. Pole,	15.04	64
	Antarctica	18.27 duplicate	60
	Godley Head, NZ	9.44	68
Volcano			
19/7/78	Iilewa, Hawaii	11.27	
		6.27 duplicate	48
20/7/78	MLC, Hawaii	12.39	46
		6.81 duplicate	51

OA, outside air; MLC, Mauna Loa Cauldera; PNW, Pacific Northwest.

^{*}Parts by volume.

around AERE Harwell, and three other air samples representing the South Pole, New Zealand and the Pacific Northwest. The results of our measurements of the concentration of CF_4 are shown in Table 1. The mean CF_4 concentration for 16 analyses of Harwell air is $63.6 \pm 13.2\%$ by volume. The mean of the other three air samples for five analyses is 64.4 ± 3.2 p.p.b. by volume. No significant difference between the two sample populations is indicated. The variability in the Harwell values is about equal to the variability obtained in repetitive and/or duplicate analyses. Accordingly the CF_4 concentration in the Southern Hemisphere and that observed for the Northern Hemisphere suggest no inter-hemispheric differences at the present level of precision of analysis.

At the same time as the CF_4 measurements of ambient air were made, two samples of volcanic emissions representative of a hot fumarole in the caldera of Mauna Loa (13,000 ft) and a much cooler vent on the north-east side of Kilauea at Iilewa (3,500 ft) were analysed. The two samples showed similar levels of CF_4 of $48.3 \pm 2.5\%$ by volume but at concentrations considerably lower than the level of 64% determined for the ambient air. The related halocarbon and other trace gas measurement data are given in Table 2.

The 25% lower level of CF_4 observed in the volcanic samples is consistent with the 17–27% lower fluorocarbon 11 and 12 levels typically observed in the Hawaiian volcanic emissions. This is interpreted to mean that the origin of both the fluorocarbon 11 and 12 and CF_4 is not from the gases being released from the fumarole's activity, but rather their presence represents entrainment of ambient air into the subterranean passages that is re-ejected into the atmosphere via the hot air plume in the chimneys associated with different volcanic vents. During the contact with the hot volcanic rocks some destruction of these materials has occurred as well as dilution. Previously Gassman had suggested that volcanic emissions may be a source of CF_4 . This may still be correct as the Hawaiian volcanic emissions studied are not typical of the active magnetic gases released

Table 2 Trace gases in Hawaiian volcanic emissions

Sample	O ₂ * (%)	N ₂ * (%)	N ₂ O (p.p.b.)†	CO ₂ (p.p.m.)†	F12 (%)†	F11 (%)†
Mauna Loa	100	100	840	3,960	211	123
Iilewa	100	100	380	360	220	140
Ambient air	100	100	330	328	275	168

*N₂ and O₂ percentages are derived from concentrations of nitrogen or oxygen in volcanic emissions over respective nitrogen or oxygen concentrations in ambient air.

†Parts by volume.

during a volcanic eruption. Also the halogen concentrations in the Mauna Loa type of volcanic emission are much lower than the halogen content of the emissions from other types of volcanoes.

The direct industrial production of CF₄ is minimal—<10 tons yr⁻¹. Therefore, its dispersive use is not expected to be a significant source of CF₄. The only other documented source of CF₄ is the electrolytic alumina reduction furnace. However, the production of CF₄ in this industrial process is not continuous, rather it is restricted to 5–10 min periods per day as the alumina content of the cell approaches depletion^{2,3}. Because of its sporadic nature, any estimate of CF₄ emissions from the alumina reduction process is subject to a very large spread in the amount of CF₄ produced and released to the atmosphere. Nevertheless, however speculative, we estimate that about 6×10^9 g yr⁻¹ of CF₄ may be emitted from the world wide 1977 production of aluminium of 1.34×10^{13} g yr⁻¹. If this is the case, then the aluminium industry is not likely to account for all of the global atmospheric burden of CF₄, which at an average concentration of 65%, approximates to 10¹² g. More information is obviously needed to establish the true contribution of CF₄ from the aluminium industry, but it is equally obvious that other sources of CF₄ need to be found.

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Trace metals in Antarctic snows since 1914

THE results of the variations of the concentrations of 12 elements including Pb, Cd, Cu, Zn and Ag in snow deposited from 1914 to 1974 at an uncontaminated site in East Antarctica are discussed here. As the composition of snow in central Antarctica is shown to be closely correlated to that in the lower atmosphere, this provides us with a valuable historical record of the composition of remote aerosols in the Southern Hemisphere. We find that Pb, Cd, Cu, Zn and Ag were already strongly enriched 60 yr ago (with respect to the composition of reference crustal and marine sources), and that both the concentrations and the enrichment factors observed in 1914 for these five elements are

comparable to the present ones. This suggests that the present background atmospheric concentrations of Pb, Cd, Cu, Zn and Ag in the Southern Hemisphere are not strongly influenced by global pollution, but are related to natural phenomena, possibly volcanism.

A recent increase in studies of the chemical composition of tropospheric aerosols in remote areas has included data^{1,2} from northern and southern regions, including the South Pole. Concentrations of heavy metals are found to be much greater than expected on the basis of the average composition of the Earth's crust and of seawater. We cannot determine whether this is due to man's increasing industrial activities by current atmospheric sampling programmes. However, we can determine the relative contributions to the composition of aerosols from natural and anthropogenic sources by analysis of impurities in successive layers of snow in polar ice sheets, as has been done for near surface layers of the Greenland ice sheet^{3–6}.

During the 1974–75 summer field season, 13 successive snow samples were collected from the surface to a depth of 5.35 m in a hand dug pit at Dome C (74°40' S, 124°10' E, elevation 3,240 m), which was at the time an uncontaminated site 600 km from the nearest scientific station and some 240 km from the nearest oversnow traverse. Samples were collected within a few weeks of the first aircraft landing at the site. The samples of the firn, about 0.7 kg each, were collected⁷ by operators clothed in clean room garb, using Plexiglass tubes with screwed caps (inside diameter 8 cm, length 42 cm, rinsed in a class 100 clean room with ultrapure acids and water) pushed directly into the snow along a scoured pit wall. The tubes were then placed in double sealed polyethylene bags and kept frozen until the analytical stage. The samples were transferred by gravity directly into Teflon bulbs inside a class 100 clean room, for preconcentration⁸ ($\times 150$) by non-boiling evaporation; this preconcentration procedure includes dissolving any solid particles by hot concentrated ultrapure hydrofluoric and nitric acids. Na, Mg, K, Ca, Fe, Al, Mn, Pb, Cd, Cu, Zn and Ag are then determined by graphite furnace atomic absorption (electrodeless discharge lamps for Pb, Cd and Zn) in the clean room. The complete procedure was calibrated⁸ by processing 80 synthetic standard solutions of various concentrations down to 10⁻¹² g/g that overlapped the Antarctic snows concentration ranges. Contamination from the field Plexiglass sampling tubes was found to be negligible for all the 12 elements⁷ by analysing ultrapure water used for an extensive rinsing of 'blank' field Plexiglass tubes. The overall analytical precision (atomic absorption and preconcentration process) calculated from the calibration curves using

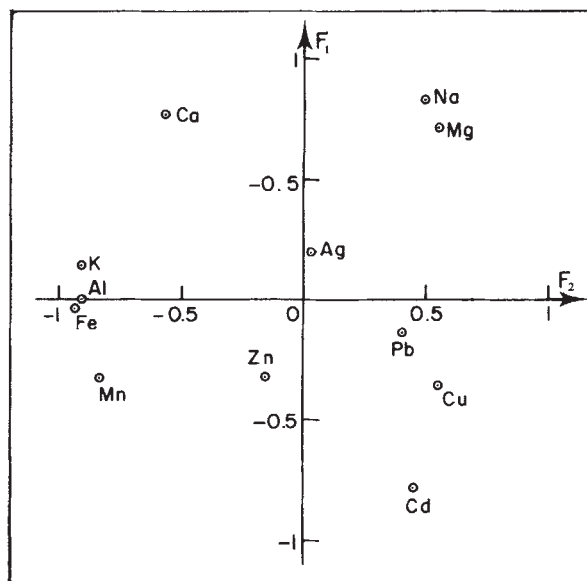


Fig. 1 Dome C, Antarctica: factor analysis. Two dimensional plot using eigen vectors F₁ and F₂.

confidence limits of 95% is of the order of 10% (30% for Cd). All data are summarised in Table 1.

The age of the snow samples collected between the surface and 2.06 m has been determined in our laboratory by various independent techniques⁹ including stratigraphy, stable isotope composition and radioactive fallout horizons from nuclear tests, which identify seasonal snow layers and make it possible to attribute specific dates to several characteristic horizons over the past 25 yr. Beyond 2.06 m, solely stratigraphic data have been used. The age of the samples ranges from 1974 to 1914, Table 1. The accuracy of these unpublished dating results (Petit, personal communication) is ± 1 yr between the surface and 2.06 m (years 1974–55); it decreases then with increasing depth to ± 5 yr at 5.35 m (year 1914).

The elements measured in this work can originate from three principal sources: the oceans, the continents and man's activities. The relative influence of these three sources at Dome C can be determined by analysing the correlation matrix of the 12 measured elements through principal components factor analysis^{10,11} (FA). FA studies this correlation matrix by diagonalising it, solving it for eigen values and vectors which are linear combinations of the original 12 elements, and taking these eigen vectors as new reference axes. The results of FA are shown in Fig. 1 for the two eigen vectors F_1 and F_2 corresponding to the two highest eigen values; these two vectors account respectively for 40.6 and 23.5% of the sample set variance. They separate three groups of elements: Na (marine reference element) and Mg (top right); Al (crustal reference), Fe, Mn, K and Ca (left); Pb, Cd, Cu, Zn and Ag (bottom right). This suggests an oceanic source for Na and Mg, a crustal continental source for Al, Fe, Mn, K and Ca, and a third source, independent from the two others, for Pb, Cd, Cu, Zn and Ag, as previously found at South Pole Station¹².

It has been suggested¹³ that in clean air conditions, the chemical composition of precipitations and of aerosols would be closely related. This is seen to be the case at the South Pole as shown by the data of Table 2. The two sets of data do not correspond to comparable time periods: this is probably unimportant as only weak seasonal variations of the concentrations are observed¹⁰ in these areas, at least in snow, but for Na and Mg. The ratio of the concentrations measured in air (expressed in 10^{-12} g per standard cubic metre) to those measured in deposited snow (expressed in 10^{-12} g/g) range from 0.4 to 1 but for K and Cd for which slightly higher ratios are obtained. Although this relationship might be influenced by various parameters, it is unlikely that marked changes happened over the past century which was characterised by rather constant climatic conditions. The variations of the trace elements content of dated snow layers in central Antarctica can therefore probably be used to reconstruct the past variations of the aerosol content of the low troposphere. Variations in concentration of all 12 elements over the period 1914–74 are shown in Fig. 2.

At Dome C there are significant variations with time for all the

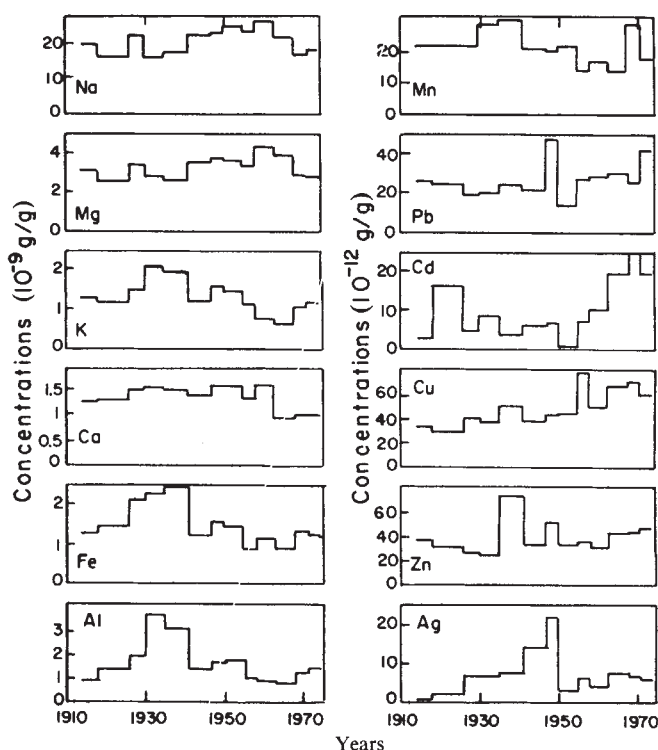


Fig. 2 Dome C, Antarctica: variations of the measured concentrations in snow between 1914 and 1974.

elements. These variations are probably not linked with short-term changes such as seasonal effects^{4,10}, as each sample integrates at least 4 yr of snow accumulation. Generally speaking, concentrations in firn of 60 yr age are similar for all the elements to the present concentrations, except perhaps for Cu, whose concentrations increase slightly ($\times 1.5$) between 1914 and 1974. Apparently, therefore, the atmospheric concentrations of the measured elements have not increased markedly, despite significant variations, since 1914. We previously found¹² an increase of the concentrations of Pb, K, Zn and Ag ($\times 2-3$) and Cu ($\times 6$) in snow between 1950 and 1974 at South Pole Station. The present data obtained at Dome C confirm this increase between 1950 and 1974 for Pb, but the increase is less pronounced for Zn, Ag and especially Cu at Dome C than at South Pole Station; no increase is detected for K. This suggests that slight local contamination associated with operations at South Pole Station, which has been occupied since 1957, may have occurred for some elements.

The absence of an increase in the atmospheric concentrations of trace elements at Dome C between 1914 and 1974 suggests that they are not significantly affected by global pollution.

Table 1 Dome C, Antarctica: measured concentrations in snow

Depth (m)	Years	Na	Mg	K	Ca	Fe	Al	Mn	Pb	Cd	Cu	Zn	Ag
				(10^{-9} g/g)						(10^{-12} g/g)			
0.10–0.48	1974–71	17.9	2.77	1.06	1.00	1.24	1.14	18	41	19	62	47	6.0
0.48–0.86	1970–68	17.0	2.82	0.93	0.99	1.36	1.25	29	25	25	72	45	6.8
0.86–1.25	1967–63	21.7	3.93	0.62	0.94	0.91	0.78	14	30	19	69	43	7.6
1.25–1.64	1962–58	26.1	4.32	0.75	1.51	1.16	0.88	17	28	8.9	50	31	4.0
1.64–2.06	1957–55	23.5	3.31	1.11	1.32	0.88	0.97	14	27	6.8	80	36	6.6
2.06–2.50	1954–50	24.8	3.61	1.40	1.55	1.46	1.81	22	15	1	45	34	2.9
2.50–2.90	1949–47	23.7	3.71	1.57	1.53	1.57	1.70	20	47	6.7	44	54	22
2.90–3.33	1946–41	21.7	3.52	1.13	1.36	1.22	1.37	21	21	6.1	39	35	14
3.33–3.75	1940–35	17.4	2.57	1.88	1.47	2.42	3.06	30	23	3.3	52	74	7.2
3.75–4.15	1934–30	16.1	2.78	2.05	1.52	2.25	3.66	29	20	8.3	38	26	6.5
4.15–4.55	1929–26	21.2	3.41	1.45	1.47	2.11	1.93	22	18	4.8	41	27	6.6
4.55–4.95	1925–18	16.2	2.48	1.12	1.27	1.47	1.39	22	24	16	29	32	2.1
4.95–5.35	1917–14	19.8	3.11	1.25	1.24	1.28	0.95	22	26	2.27	34	38	0.2

Historical records of some trace metals discussed here have already been published for Greenland in the Northern Hemisphere³⁻⁶. No trend was found for Hg⁵ and Cd^{4,6}, while Pb^{3,4} and Zn⁴ concentrations were shown to have increased by a factor of two to three since the beginning of the century. There was no clear results for Cu⁶. Our results therefore indicate that industrial pollution has had no significant effect on the background composition of the atmosphere of the Southern Hemisphere, although it seems to have some effect on the background content of some heavy metals (Pb, Zn) in the Northern Hemisphere atmosphere. This is in good agreement with the fact that more than 90% of anthropogenic aerosols are emitted in the Northern Hemisphere¹⁵, and that the Equator is a barrier for tropospheric aerosols.

The interesting features, shown in Fig. 2, are the high measured concentrations of Al, Fe, Mn and K (elements of crustal continental origin according to FA) between 1925 and 1940 approximately. This could be explained by an enhancement of the meridional circulation between 1925 and 1940 in the Southern Hemisphere, resulting in an increase of the transport fluxes of crustal material from mid latitude continents to Antarctica. We have no literature values on the historical variations of the circulation patterns in the Southern Hemisphere, but it has been suggested¹⁶ that, in the Northern Hemisphere, they were different between this time period and the remaining other years of the 1900-68 period.

The variation with time of the enrichment factors with respect to bulk seawater (EF_{sea}) for Mg, and with respect to the mean crustal composition¹⁷ (EF_{crust}) for K, Ca, Fe, Mn, Pb, Cd, Cu, Zn and Ag are shown in Fig. 3. The reference elements are respectively Na and Al. The precision is better than 20% for all the elements except for Cd (40%). Calculated EF_{sea} values are close to unity for Mg, confirming that the sea is the likely source for this element. EF_{crust} values near unity are obtained for K, Ca, Fe and Mn suggesting again a crustal continental source for all these elements.

On the other hand, high EF_{crust} values ranging from one to four orders of magnitude are observed for Pb, Cd, Cu, Zn and Ag. This supports the FA data showing an independent source

Table 2 South Pole station: comparison of the chemical composition of deposited snow with that of local atmospheric aerosols

Element	Concentrations	
	Aerosols* (10^{-12} g/standard cubic metre)	Snow† (10^{-12} g/g)
Na	3,300 ± 1000	7,700 ± 140
Mg	720 ± 430	1,300 ± 130
K	680 ± 140	370 ± 70
Ca	490 ± 200	510 ± 190
Fe	620 ± 230	760 ± 90
Al	820 ± 380	1,070 ± 250
Mn	13.5 ± 3.5	18 ± 4.5
Pb‡	{ 27 ± 10 76 ± 40	40 ± 11
Cd	18 ± 13	3.8 ± 2.8
Cu	29 ± 17	47 ± 1.4
Zn	33 ± 20	6.8 ± 7.8
Ag	3.4 ± 3.6	5.5 ± 0.07

* From ref. 14. The results are the average of five sampling periods of nine days between 16 December 1974 and 4 February 1975. The error listed is the standard deviation derived from the dispersion of the concentrations for the five sampling periods.

† From ref. 10. The results are the mean concentrations measured in four snow samples integrating the years 1973 and 1974. The error listed is the standard deviation derived from the dispersion of the concentrations for the four samples.

‡ The two values for aerosols correspond to two sets of samples collected by using different sorts of filters (see ref. 14).

for these last elements. For Pb, Cd, Cu and Zn, the EF_{crust} decrease more or less sharply from 1914 to 1930; they remain then almost constant between 1930 and 1950, and then increase from 1950 to 1974. There are large variations for Ag, but the mean values between 1914 and 1930 are not very different from those between 1950 and 1974. It seems that despite large variations with time, the enrichments factors for the heavy metals are as high in 1914 as the ones observed in 1974. This again suggests that these high enrichments observed for heavy metals in Antarctic snows, and therefore for the Southern Hemisphere background aerosols, are not significantly influenced by global pollution, but are linked with natural phenomena.

The possible natural sources or mechanisms responsible for these very important enrichments of heavy metals are still being discussed^{1,2,4,18}. The emission into the atmosphere of heavy metals from the ocean surface microlayer^{19,20} and from plants^{21,22} has been described and could be very significant. However, the most likely source could be volcanism. Little information is available on the chemical composition of volcanic aerosols, but it seems that they are strongly enriched in Pb, Cd, Cu, Zn and Ag^{23,24}, with enrichment factors close to those determined at Dome C^{23,24}. The background fallout fluxes of these five elements for the Southern Hemisphere calculated²⁵ from our Dome C data (by assuming as a first crude approximation an homogeneous deposition pattern) correspond approximately to emission fluxes²⁴ from about 10 volcanoes similar to Mt Etna in Italy. Moreover, the historical record of the stratospheric burden of volcanic aerosols²⁶ shows that after decreasing from 1900 to 1920, and being low between 1920 to 1945, this burden increased after 1945. These time variations are similar to observed changes of concentrations shown in Fig. 2 and enrichment factors shown in Fig. 3 for Pb, Cd, Cu, Zn, but not for Ag. Although it has been shown²⁷ that remote volcanic aerosols can reach the Antarctic ice cap, more detailed information on volcanic sources (chemical composition, fluxes) and analyses of snow samples covering a much longer time period including more particularly the years 1850-1910 which were characterised by the two powerful eruptions of Krakatoa (1883) and Santamaria (1903) are needed to confirm if volcanoes are

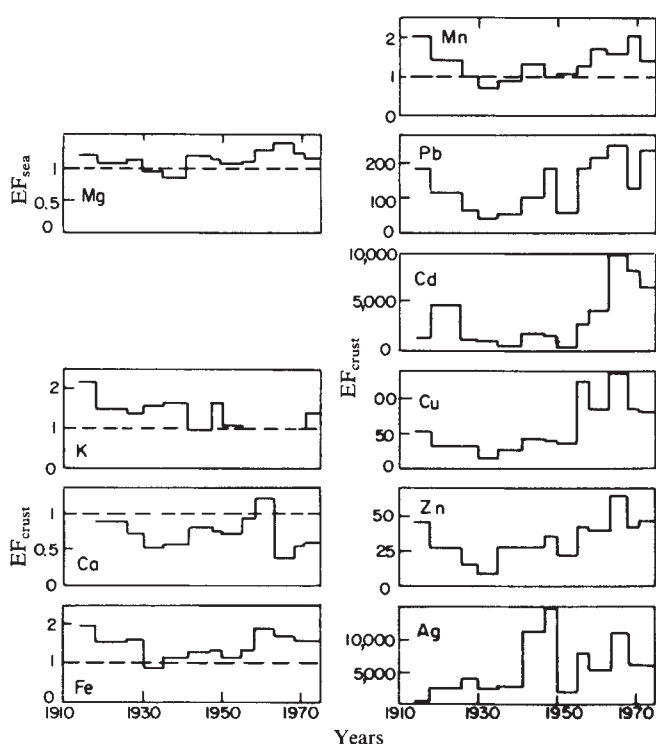


Fig. 3 Dome C, Antarctica: variations of the enrichment factors between 1914 and 1974.

really responsible for the past and present day atmospheric concentrations of heavy metals.

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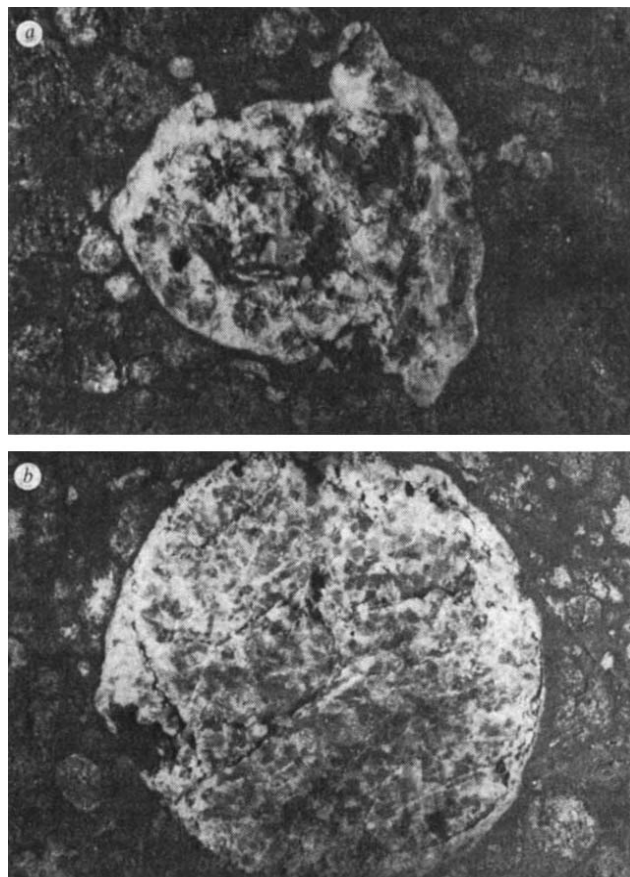


Fig. 1 Photograph of two Allende inclusions from which chips were taken for ^{40}Ar – ^{39}Ar analysis. (Sample 17(a); sample 18(b)).

would be due to an enhanced $^{40}\text{K}/^{39}\text{K}$ isotopic ratio produced in the explosive carbon burning shell of the supernova⁷. In another, controversial interpretation these ages could have chronological significance, as here the presolar grains are relicts from various old stellar nucleosynthetic and condensation processes unrelated to the formation of the Solar System^{8–10}.

Sample 17 was a 15.1 mg interior chip of an irregular white cavernous inclusion (Fig. 1a). Binocular inspection revealed the presence of at least three different mineral phases: dark grey and clear crystals (up to 1 mm) were closely packed in a sugary white matrix material. The inclusion was surrounded by an 80–100 μm wide, extremely fine-grained rim locally enriched in spinels.

Sample 18 was a 40.9 mg interior chip of a round white inclusion (Fig. 1b) with a diameter of ~ 11 mm, which also had a core and a rim (0.2 mm). The core consisted of dark crystals (0.5 mm) embedded in a light grey crystalline matrix. Microscopic investigation showed a mineral composition typical of type B inclusions². The core of the inclusion consists of prismatic xeno- and hypidiomorphic clinopyroxene crystals (80 μm –1 mm, majority 200–500 μm). Melilite and anorthite are subordinately present, melilite as xeno- or hypidiomorphic, sometimes elongated grains (350 μm –2 mm), anorthite as lath-shaped twinned crystals. Spinel occurs either as hypidiomorphic isolated grains (2–25 μm), poikilitically enclosed in pyroxene, melilite, and anorthite, or as round aggregates (30–160 μm) of minute crystals. Inclusions of opaque minerals dispersed in pyroxene and melilite are common. The rim of the inclusion, 80–100 μm wide, consists of extremely fine-grained crystalline material, partly enriched in spinels (5–15 μm). Some pyroxene crystals show deformation lamellae. More detailed mineralogical as well as chemical studies of these inclusions are in preparation.

The irradiation, argon extraction, purification, and data

Gerontology of the Allende meteorite

In the Allende meteorite several elements are found to have an isotopic composition that cannot be due to radioactive or spallation or fractionation processes¹. These isotope anomalies are mostly confined to white inclusions enriched in refractory elements² (Ca–Al-rich inclusions) and are thought to be introduced into the Solar System by precondensed grains³. We have previously reported an anomalously high K–Ar age of a composite sample of five white inclusions from the Allende meteorite⁴; no direct verification of this lone-standing result of 4,800 Myr was possible because, with the exception of a small chip of one of the inclusions used for a thin section, the material was fused in the ^{40}Ar – ^{39}Ar experiment. We therefore began a search for old material in other Ca–Al-rich inclusions. Three different samples from a new type of inclusion proved to be no older than most of Allende's constituents, 4,510 Myr (ref. 5). A small white inclusion gave a well developed $^{40}\text{Ar}/^{39}\text{Ar}$ plateau over almost the entire ^{39}Ar release, with an age of $4,500 \pm 20$ Myr (ref. 6). We report here on the ^{40}Ar – ^{39}Ar analysis of two further coarse-grained Allende inclusions that showed ages in excess of 4,550 Myr.

In the most widely accepted theory a supernova triggered the collapse of the solar nebula^{3,7} and the anomalously high ages

analysis procedures are identical to those previously published¹¹, except that the atmospheric argon was not removed before the ^{40}Ar - ^{39}Ar experiment by preheating.

The results of the argon analysis are given in Table 1, at the same stage of data reduction as in ref. 11. For the apparent ages (I), a correction for trapped ^{40}Ar with the conventional ratio ($^{40}\text{Ar}/^{36}\text{Ar}$)_{trapped} = 1 ± 1 was applied. To take into account atmospheric contamination, minimum ages (II) were calculated after subtraction of $295.5 \times ^{36}\text{Ar}_{\text{trapped}}$ (that is, in the atmospheric ratio) from ^{40}Ar . These ages are the basis of our present discussion. Age spectra are shown in Fig. 2.

Absolute concentrations are 155 p.p.m. K, 12% Ca and 70 p.p.m. K, 14% Ca for sample 17 and 18, respectively. In the reactor irradiation ^{38}Ar is produced from Cl present in small amounts¹² and thus nominal $^{38}\text{Ar}/\text{Ca}$ exposure ages in these inclusions are too old compared with the known cosmic ray exposure time of Allende of 5 Myr (ref. 13). Note, however, that ^{38}Ar from Cl is small in the 1,130–1,380 °C fractions of sample 17, which yields a weighted average exposure age of 3.9 Myr (production rate 2.8×10^{-3} ccSTP $^{38}\text{Ar}_{\text{C}}$ per g_{Ca} Myr).

The age spectra obtained from both samples are rather similar. Taking the minimum apparent ages, we observe broad plateaus extending over the first 50–60% of ^{39}Ar released. At high extraction temperatures, beginning at 1,070 and 1,300 °C, respectively, the apparent ages become older by about 400 Myr, compared with the plateaus.

The two-step patterns of the age spectra are reflected in the K/Ca variations. K/Ca of the plateau fractions is relatively stable, most pronouncedly so in sample 17, indicating that over a wide temperature regime the same mixture of phases is degassed. Low K/Ca ratios at higher temperatures are associated with high ages, the opposite of what is expected from ^{39}Ar recoil¹⁴.

Two-step ages patterns have also been previously encountered in lunar highland breccias^{11,15,16}. There the significance of both parts of the spectra had been confirmed by spotwise laser ^{40}Ar - ^{39}Ar dating^{17,18}. The difference between the results of

those lunar studies and of Allende samples 17 and 18 is the >4,500 Myr age of the latter and the 'normal' ages of the former. In addition to the samples reported here, we irradiated and analysed a sample of lunar highland breccia 73215, 170, 6, a pink spinel-bearing troctolitic clast (O. B. James, personal communication), which below 1,050 °C yields a plateau age of $3,940 \pm 70$ Myr in agreement with the formation age of the breccia of 3,870 Myr (ref. 19). At 1,400 °C half of the ^{39}Ar is released with an apparent age of $4,460 \pm 40$ Myr. This age is about the same as obtained for other early lunar differentiates^{15,20}; it may even be the oldest one. The age of this clast fits perfectly into the chronological framework established for breccia 73215 (refs 11, 19, 21). It also demonstrates that the >4,500 Myr ages of our two Allende inclusions are not due to some kind of a calibration error. Instead, the new results support the 4,800 Myr K-Ar age previously reported. The nominal K-Ar ages of samples 17 and 18 are $5,120 \pm 60$ Myr and $5,080 \pm 50$ Myr, and the plateau ages are $4,980 \pm 60$ Myr and $4,900 \pm 30$ Myr, respectively. A high-temperature phase must be responsible for the still higher apparent ages of the 1,130 or 1,300 °C fractions, averaging $5,400 \pm 100$ Myr.

The ratio of 'excess' ^{40}Ar (over that produced since 4,550 Myr) to K is 0.033 ccSTP per g for both samples. It is unlikely that this excess ^{40}Ar migrated from the K-poor matrix (200 p.p.m. Jessberger *et al.*, in preparation) into the inclusions. Not only is there no precedent for such a migration, but it would also have to occur to precisely the right degree to balance the different K concentrations.

It has long been argued that the isotopic anomalies and some other peculiar features of the Ca-Al-rich inclusions were introduced into the Solar System by presolar grains^{8,22-25}. Two principal origins of the presolar grains have been proposed. According to Clayton⁹, they represent interstellar dust from various sources. Such dust, if not outgassed, should have a mean K-Ar greater than that of the Solar System. In that picture¹⁰, the anomalously old ages are real and indicate the survival of

Table 1 Amounts and isotopic composition of Ar released from two coarse-grained white inclusions of Allende after experimental corrections. Apparent ages II are calculated after subtraction of $295.5 \times ^{36}\text{Ar}_{\text{T}}$ from ^{40}Ar to account for atmospheric contamination

a Allende no. 17 (15.1 mg)							
Extraction temperature (°C)	^{40}Ar (10^{-8} ccSTP per g)	$^{36}\text{Ar}/^{40}\text{Ar}$ ($\times 10^5$)	$^{37}\text{Ar}/^{40}\text{Ar}$ ($\times 10^3$)	$^{38}\text{Ar}/^{40}\text{Ar}$ ($\times 10^5$)	$^{39}\text{Ar}/^{40}\text{Ar}$ ($\times 10^5$)	Apparent age (10^3 Myr)	
						I	II
500	117	301 ± 6	32 ± 1	102 ± 5	99 ± 4	7.68 ± .08	4.96 ± .42
580	79	215 ± 7	55 ± 1	93 ± 5	283 ± 13	5.83 ± .08	4.60 ± .16
660	140	139 ± 4	54 ± 1	121 ± 4	374 ± 9	5.35 ± .04	4.97 ± .06
740	114	107 ± 2	77 ± 3	133 ± 4	436 ± 6	5.09 ± .02	4.97 ± .04
820	99	111 ± 3	156 ± 2	146 ± 5	439 ± 11	5.08 ± .04	4.99 ± .06
890	116	146 ± 2	162 ± 2	227 ± 5	408 ± 10	5.21 ± .04	5.21 ± .04
950	99	158 ± 7	133 ± 1	383 ± 8	470 ± 16	4.97 ± .06	4.97 ± .06
1,010	165	231 ± 5	116 ± 1	578 ± 7	470 ± 7	4.97 ± .03	4.97 ± .03
1,070	93	286 ± 3	286 ± 3	535 ± 14	377 ± 8	5.34 ± .04	5.34 ± .04
1,130	945	64 ± 4	1,104 ± 1	69 ± 4	335 ± 4	5.54 ± .02	5.43 ± .04
1,200	140	278 ± 15	3,327 ± 13	232 ± 13	227 ± 13	6.21 ± .10	5.25 ± .24
1,280	31	498 ± 36	9,540 ± 40	221 ± 34	411 ± 46	5.19 ± .19	1.46 ± 1.48
1,380	52	667 ± 47	14,050 ± 70	598 ± 52	130 ± 40	7.20 ± .59	2.67 ± 5.65
Total	2,189	156 ± 2	1,209 ± 2	188 ± 3	344 ± 4	5.50 ± .02	5.12 ± .06
b Allende no. 18 (40.9 mg)							
450	298	339 ± 1	—	75 ± 2	15 ± 1	11.01 ± .06	4.52 ± 2.24
580	116	313 ± 3	9 ± 1	82 ± 3	80 ± 6	8.06 ± .14	4.47 ± .50
670	145	290 ± 3	15 ± 1	96 ± 2	109 ± 2	7.50 ± .04	4.97 ± .16
750	124	243 ± 4	41 ± 1	117 ± 2	215 ± 3	6.31 ± .03	4.90 ± .09
830	78	193 ± 3	76 ± 1	237 ± 6	370 ± 4	5.37 ± .02	5.13 ± .06
900	45	224 ± 9	112 ± 1	629 ± 3	471 ± 11	4.96 ± .04	4.96 ± .04
970	39	312 ± 7	160 ± 2	1,027 ± 10	490 ± 13	4.90 ± .04	4.90 ± .04
1,040	45	372 ± 9	225 ± 2	1,359 ± 11	492 ± 11	4.89 ± .04	4.89 ± .04
1,110	37	404 ± 5	422 ± 2	1,487 ± 9	489 ± 9	4.90 ± .03	4.90 ± .03
1,190	101	355 ± 6	1,019 ± 3	990 ± 6	514 ± 5	4.82 ± .02	4.82 ± .02
1,300	318	710 ± 22	6,968 ± 7	1,760 ± 20	372 ± 21	5.37 ± .10	5.37 ± .10
Total	1,345	401 ± 5	1,761 ± 2	685 ± 5	250 ± 5	6.05 ± .04	5.08 ± .05

non-degassed K-bearing grains 'from the beginning of the Universe' through the turbulences of the Ca-Al-rich inclusion-forming processes. Irradiation of the presolar grains with cosmic ray particles before formation of the Solar System is proposed by Clayton's model. The absence of large amounts of spallation ^{38}Ar , however, does not necessarily invalidate this model, as the size of interstellar grains²⁶ is not sufficient to stop (and hence trap) the recoiling spallation products within the boundaries of the grains.

According to the second model, which is not in all aspects contradictory to the first, the presolar grains condensed in a late supernova that triggered the collapse of the solar nebula^{3,7}. In this model apparent K-Ar ages can result from an isotopic enhancement of ^{40}K . Note here that K-Ar ages are usually not based on $^{40}\text{Ar}/^{40}\text{K}$ ratios, as the principle of the method would require, but in the case of the ^{40}Ar - ^{39}Ar method they are calculated from $^{40}\text{Ar}/^{39}\text{K}$ ratios, which are converted to the proper ratios by assuming that the sample has the normal Solar System ratio of $^{40}\text{K}/^{39}\text{K}$. As calculated by Cameron and Truran⁷, the explosive carbon burning phase of supernovae produces a $^{40}\text{K}/^{39}\text{K}$ ratio that is 70 times the average Solar System ratio 4,600 Myr ago.

Other anomalies like the ^{48}Ca enhancement²⁷ are also attributed to the explosive carbon burning phase as are FUN anomalies^{28,29}. An apparent K-Ar age of 5,100 Myr corresponds to a ^{40}K enrichment of 35%, whereas an age of 5,400 Myr as measured in the high temperature fractions of samples 17 and 18, requires a 60% enrichment of ^{40}K relative to the Solar System value of $^{40}\text{K}/^{39}\text{K}$. These large enrichment factors, however, do not imply extraordinarily large numbers of

extra ^{40}K atoms. The magnitude of this anomaly is only 1/50 of the excess ^{48}Ca atoms in inclusion EK 1-4-1 (ref. 27).

Unfortunately, the possible presence of a ^{40}K isotopic anomaly does not clearly distinguish between the two models, as both allow for ^{40}K enhancements. However, Clayton¹⁰ predicts an additional ^{41}K anomaly in his model. This letter does not attempt to discuss the merits or problems of both models.

Up to now three Ca-Al-rich inclusions have been analysed for their K isotopic composition and have been found to have normal ratios^{30,31}. These negative results, however, do not preclude samples 17 and 18 from having anomalous K. Note that so far, FUN anomalies of Mg, O (refs 28, 32) have only been encountered in two inclusions of Allende out of some 50 searched. Thin section studies of the parent inclusion of sample 18 revealed the peculiar feature of magnetite preceding the iron-phase condensation (A. El Goresy, personal communication). Samples 17 and 18 may represent new examples of presolar FUN material.

The experimental verification of gas retention ages older than the accepted age of the Solar System in itself should not be interpreted as evidence for the survival of presolar grains—although the 'ages' are highly suggestive—until the carrier material of the extra ^{40}Ar is identified and further isotopic evidence obtained.

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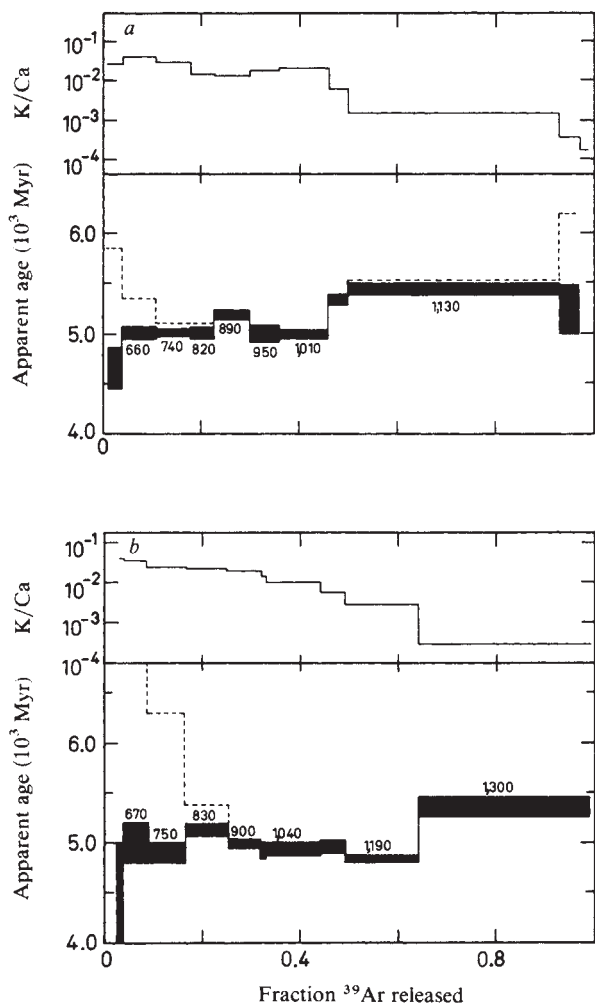


Fig. 2 K/Ca and apparent age spectra of two white Allende inclusions. The broken lines in the age spectra represent the apparent ages without correction for atmospheric contamination.

Continental geotherms during the Archaean

PRESSURE and temperature data from Archaean terrains have been used to test whether heat production during the Archaean at about three times the present rate resulted in correspondingly steeper continental geothermal gradients¹⁻⁶. The rocks considered here lie mostly in the range of medium pressure metamorphism, recording pressures of 7–13 kbar at temperatures of 600–900 °C (ref. 2). These pressures, with the underlying, normal, thickness of crust imply great thickening of continental crust during the Archaean.

Although these temperatures and pressures may record conditions during a phase of crustal generation—presumably dominated by igneous activity—the time for thermal equilibration of such a province is short (~50 Myr: for example, oceanic lithosphere) and unless the ages of the mineral parageneses can be tied in with this accuracy to phases of crustal generation, the crustal thickening and P - T data may result from a later and quite separate metamorphic episode.

By analogy with the behaviour of Phanerozoic terrains, most of the exhumation of these rocks must have occurred in the first few 100 Myr after the episode of crustal thickening. In these circumstances a P - T estimate represents only a point in P - T -time space and it is of little use, therefore, to try to fit it to any steady-state geotherm^{7,8}, particularly to one for normal crustal thickness.

However, it will be shown that if the time-dependent nature of the geotherm is accounted for, these data are still useful in that they give an upper limit to the geothermal gradient in thickened Archaean crust^{5,6}. This is self-evident if the overthick crust was produced by magmatic intrusion, but it needs to be demonstrated if the thickening was not accompanied by extensive magmatism.

In the first case, the intrusive terrain would have cooled rapidly so that the P - T data reflect closely the magma-steepened geotherm, even when erosion is rapid⁷. If, however, the overthick crust was produced by over- or under-thrusting this will not necessarily be true. The common feature of these forms of crustal thickening is the lateral injection of cool crustal material, rather than the vertical emplacement of hot fluid. This results in the establishment of geotherms with dT/dP less than equilibrium ones, and their persistence for several tens of Myr (ref. 9); similar conditions apply if the overthick crust is produced by rapid sedimentation.

Erosion can considerably steepen the geotherm, however, and this, with continuing movement towards equilibrium during erosion, may mean that rocks buried deeper than about 30 km will not begin to cool until they have been decompressed by several kbar (refs 7, 10).

This point is demonstrated in Fig. 1 for two hypothetical terrains in which there is a linear gradient with the rock considered (initially buried at 40 or 60 km) at 900 °C. These profiles are then eroded at a linear rate¹¹ so that the rocks reach the surface in 50 or 200 Myr. The P - T paths for these rocks are shown in Fig. 1; when the rock is exhumed from 60 km between 80 and 90% of its cooling occurs in the last 30 km of its ascent and when it is exhumed from 40 km, the last 20 km of ascent shows 70–85% of the cooling. The portions of the P - T paths characterised by nearly isothermal decompression would be extended to lower pressures if there were radiogenic elements in the pile, as heating would continue during the ascent (see refs 7, 10). Also plotted in Fig. 1 are P - T data for the metamorphism of Archaean granulites summarised by Tarney and Windley²; a mean crustal density of 2.85 Mg m⁻³ is assumed in converting pressures to depth.

If the initial geotherms (dotted lines in Fig. 1) represent thermal profiles at, say, 70% of the temperatures which would be supported at equilibrium, then the dashed lines (E) show this equilibrium profile; probable P - T data from these rocks would lie close to (40 km burial) or to the high temperature side (60 km burial) of these equilibrium profiles. These arguments suggest that, whether the Archaean P - T data result from thickening of the crust by magmatism or from compression of cooler crustal material, they almost certainly represent temperatures as high as, or higher than, those which could be maintained at equilibrium in the thickened Archaean crust.

To estimate a 'normal' Archaean continental geotherm from the P - T data, thermal profiles for the thickened crust are calculated. It is assumed that a quantity of radiogenic heat production q_A , is distributed—either uniformly, or exponentially decreasing with depth on a scale length of 10 km—through the burden overlying the rocks which now yield the P - T data. The temperatures are sustained by this heating and a deep-

seated contribution, q_* , from the lower portions of the crust and the mantle. The conductivity, K , is assumed constant throughout. A temperature of 900 °C at 40 km is taken as representative of the granulite P - T data; from the arguments given above this temperature is likely to be no lower than the equilibrium temperature at that depth in the overthick crust.

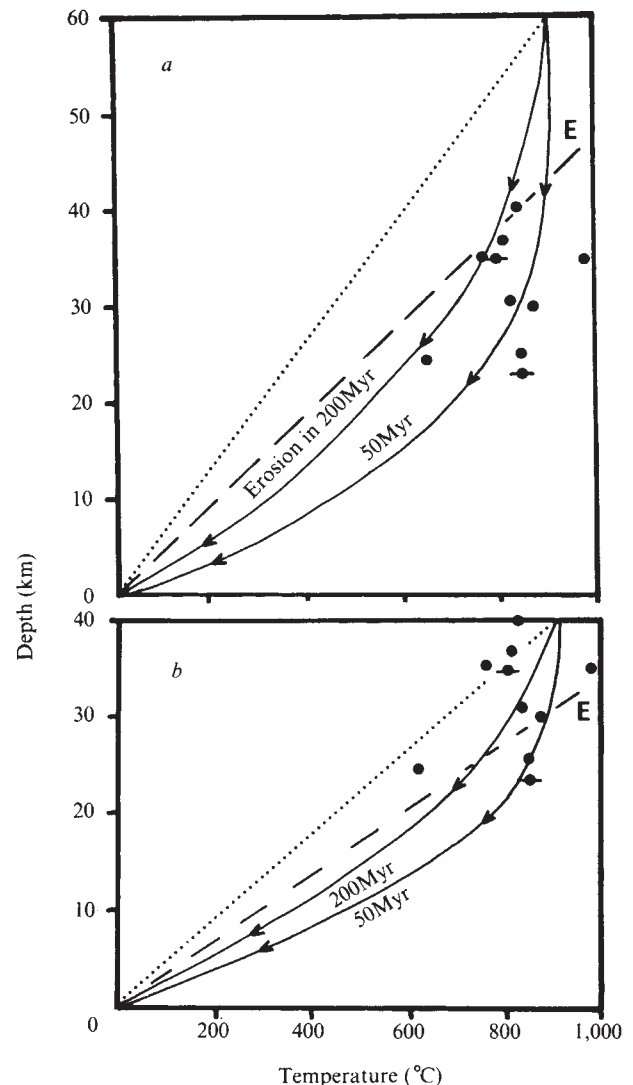


Fig. 1 P - T paths for rocks buried at depths of 60 km (a) and 40 km (b) in terrains of initially uniform thermal gradient such that they are at 900 °C at these depths. The rocks are subsequently exposed by erosion at a uniform rate over 50 or 200 Myr. Temperatures are calculated from Carslaw and Jaeger¹¹. ● Indicate P - T estimates from Archaean terrains²; pressure and temperature ranges are plotted at their mid-points; and ○ indicate that the pressure estimates are minima. Dotted lines (E) are 'equilibrium' geotherms if initial gradients represent only 70% of equilibrium temperatures.

An upper limit on the mantle contribution to Archaean continental geothermal gradients may be obtained by assuming that these temperatures were maintained without the help of radiogenic heating in the crust. The average crustal thermal conductivity is unlikely to have been greater than 4 W m⁻¹ K⁻¹ and this yields a limit of 90 mW m⁻² for the Archaean mantle contribution to surface heat flow. In these conditions the temperature at the base of 35-km thick crust would be around 800 °C.

The heat production distribution in the overburden to the rocks preserving Archaean P - T data is, naturally, indeterminable and no more rigorous limit may be placed on q_* from these

data. However, a plausible lowest limit on the radiogenic heat production in Archaean continental rocks may be obtained from measurements on the Th- and U-depleted pyroxene granulites from Australian Archaean terrains¹² and from measurements on high grade rocks in the Canadian Shield (see ref. 13). These rocks, after allowance for the decay of heat producing elements since the Archaean, would generate at least $1 \mu\text{W m}^{-3}$, or a total of 40 mW m^{-2} through 40 km of overburden.

Table 1 shows the value of q_* required to maintain 900°C at 40 or 60 km for a given value of q_A -distributed exponentially or uniformly through the 40 or 60 km of cover. Crustal radiogenic heat production at 40 mW m^{-2} over 40 km of cover requires q_* of $70\text{--}80 \text{ mW m}^{-2}$; some of this must come from the subjacent crust and if the estimate of $1 \mu\text{W m}^{-3}$ for heat generation in the Archaean continental crust is reasonable, this portion would be $20\text{--}30 \text{ mW m}^{-2}$.

Lower estimates of q_* would be obtained if it could be shown that exhumation from more than 40 km was responsible for the generation of the P - T data under discussion. Table 1 shows that q_* of $40\text{--}60 \text{ mW m}^{-2}$ is required for the conditions described above if the rocks originated at 60 km rather than 40 km. Such rocks would follow the kind of P - T - t path of Fig. 1a and would perhaps show a record of progressive metamorphism during the early part of the path, when decompression was nearly isothermal.

Table 1 Values of q_* required to sustain temperatures of 900°C at 40 and 60 km for different values of K and q_A (see text)

Heat generation distributed uniformly						
q_A (mW m^{-2})	Depth of burial 40 km			Depth of burial 60 km		
	Conductivity ($\text{W m}^{-1} \text{K}^{-1}$)					
	2	4	6	2	4	6
0	45	90	135	30	60	90
20	35	80	125	20	50	80
40	25	70	115	10	40	70
60	15	60	105	—	30	60
80	5	50	95	—	20	50

Heat generation distributed exponentially						
	2	4	6	2	4	6
0	45	90	135	30	60	90
20	40	85	130	27	57	87
40	35	80	125	23	53	83
60	30	75	120	20	50	80
80	25	70	115	17	47	77

These lower estimates of q_* would result in temperatures of $400\text{--}700^\circ\text{C}$ at the base of 35 km thick crust after the overburden was removed.

In conclusion, P - T data from high grade Archaean terrains represent temperatures reached during the exhumation of the rocks from an overthick crust and are probably not lower than equilibrium profiles for this thickened crust. At present the best upper limit which may be placed on the heat flow from the Archaean mantle from these data is 2–3 times present-day sub-continental values, and in this respect the P - T data offer no improvement in constraints on Archaean geothermal regimes over those from general observations of continental integrity in the Archaean: if a craton was formed of rocks of low radiogenic heat production and high conductivity, the base of its crust need not have approached melting temperatures even with mantle heat flow of 90 mW m^{-2} . On the other hand, mantle heat flow this high is not demanded by the observations; the P - T data could well represent a thermal regime in which the heat flow from below the continental crust was 40 mW m^{-2} or less.

It would be possible to lower the upper bound on Archaean continental geothermal gradients if reliable information were obtained on the distribution of heat-producing elements in the Archaean crust and on the P - T -time paths followed during the exhumation of high grade Archaean rocks.

The lower geothermal gradients permitted by the P - T data would be consistent with the view of Archaean tectonics in which the continental thermal regime was similar to that of present day and most of the additional heat was lost from the Earth's interior by a faster rate of creation of oceanic lithosphere^{1,6}.

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Uranium series dating of travertine from archaeological sites, Nahal Zin, Israel

At present, absolute dates of occupation can readily be determined for prehistoric sites no older than about 40,000 yr BP using conventional ^{14}C techniques. Ages of older sites can be obtained with the K-Ar method, but only where volcanic deposits are interstratified with the archaeological deposits. The majority of Middle and Lower Palaeolithic sites contain no volcanic deposits. However, many of these sites are associated with synchronously deposited beds of calcium carbonate, occurring either as tufa mounds left by now extinct springs, or as layers of travertine (speleothem) in the mouths of formerly inhabited caves. We show here that such calcareous deposits can be dated by the ^{230}Th - ^{234}U method, as long as they are relatively impermeable and free of detritus; ages in the range 5,000–400,000 yr BP are obtainable with a precision of 5–10%. When the carbonate is deposited, it invariably contains traces of uranium but essentially no thorium. Its age can be obtained from the extent to which ^{230}Th has grown into radioactive equilibrium with its parent ^{234}U . The technique of radiochemical analysis is given in ref. 1. The ^{230}Th - ^{234}U method has been found to be less reliable for the dating of molluscs²; although it has also been applied to the dating of bones³, the method has often yielded contradictory results, probably attributable to continuous diffusion of radionuclides through the bone during burial⁴. Some permeable, very fine-grained tufas have also given poor results in earlier studies⁵; however, careful choice of dense, impermeable, coarsely crystalline travertines generally yields dates that are mutually consistent and coherent with other geochronological tests⁶.

We have applied this method to the dating of travertines found in association with artefacts of Middle and Upper Palaeolithic typology from sites in Nahal Zin in the north-central Negev Highlands, Israel. These open-air sites have been

described by Marks *et al.*⁷. Lying on the floor of Nahal Mor (Fig. 1) is a large block of travertine in which there occurs a layer of artefact-bearing breccia. The artefacts include a few blades, flakes and a single blade core. The block has evidently fallen from a massive deposit of travertine seen clinging to the steep wall of the canyon about 30–35 m above the present valley floor. The blade core is particularly interesting because it exhibits hard hammer technique, an absence of edge regularisation, platform faceting only on the margin adjacent to the face of the core, and the removal of sizeable blades. These artefacts and the core in particular resemble material found in level 4 of site Boker Tachtit (D 101)⁸ located in an alluvial terrace ~1.8 km downstream from the travertine block. Artefacts from this site represent the transition from Middle to Upper Palaeolithic typology. Charcoal from level 1 of site D 101 gave ages of >45,500, >35,000 and $45,000 \pm 2,400$ yr BP; the stratigraphy of the site suggests that there is very little difference between the ages of levels 1 and 4.

Travertine samples from above and below the breccia layer exhibit very uniform $^{234}\text{U}/^{238}\text{U}$ ratios and uranium concentrations (Table 1), suggesting deposition over a relatively short

time. There is no significant difference between the ages of samples from above and below the breccia stratum; pooling all the data, we conclude that the artefacts were deposited at $46,500 \pm 2,900$ yr BP. This agrees satisfactorily with the ^{14}C estimates at D 101. Another adjacent fallen block of travertine gave an age in the same range. The alluvial terrace on which site Boker Tachtit was deposited, although largely removed by down-cutting of Nahal Mor, can be traced up the canyon to a point 20–30 m below the travertine mass from which the dated blocks must have fallen. Water from this now inactive spring may have been used by the occupants of Boker Tachtit, who chanced to leave the artefacts found embedded in the travertine.

About 5 km to the south-east, near the head of the Nahal Aqev (Fig. 1), lies a dissected mound of travertine marking the site of a former spring (Fig. 2). A layer of colluvial sediment interlaminated between the two outermost layers comprising this travertine mound contains several artefacts of Levallois technique⁹. They resemble artefacts found in site Nahal Aqev (D 35), 150 m NNE from the fossil spring. The artefacts from site D 35 are ascribed to the early Mousterian¹⁰ and resemble Copeland's¹¹ Levantine Mousterian phase 1. These artefacts

Table 1 Isotopic and age data for travertines from Avdat/Aqev area

Sample no.	Description	Travertine blocks in Nahal Mor				Age (10^3 yr BP)
		$^{234}\text{U}/^{238}\text{U}$	U (p.p.m.)	$^{230}\text{Th}/^{234}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$	
76NZ3-2	Below artefact layer	0.940 ± 0.032	3.14	0.372 ± 0.014	80.8	50.7 ± 2.5
76NZ3-2A	Below artefact layer	0.950 ± 0.019	3.04	0.362 ± 0.012	44.1	49.0 ± 2.0
76NZ3-1	Below artefact layer	0.938 ± 0.030	3.81	—*	—	—
76NZ4B-2A	Above artefact layer; upper lamina	0.915 ± 0.023	2.45	0.318 ± 0.020	>1,000	41.8 ± 3.1
76NZ4B-3	Above artefact layer; middle lamina	0.955 ± 0.029	2.91	0.206 ± 0.013	>1,000	25.0 ± 1.8
76NZ4B-4a	Above artefact layer; lower lamina	1.161 ± 0.030	4.71	0.344 ± 0.077	>1,000	45.2 ± 12.5
76NZB-4a	Above artefact layer; lower lamina	0.937 ± 0.025	2.41	0.352 ± 0.021	56.4	47.3 ± 3.6
76NZ4A-1	Above artefact layer	1.054 ± 0.062	1.35	0.380 ± 0.030	9.9	$45.2 \pm 5.2^\dagger$
76NZ5-2	Another travertine block, 0.5 m away from NZ4	0.942 ± 0.043	2.25	0.344 ± 0.025	68.8	46.0 ± 4.2
	Average omitting 76NZ4B-3	0.977 ± 0.074	2.90 ± 0.96			46.5 ± 2.9
Nahal Aqev, fossil spring‡						
76NZ6e-1	layer A	0.924 ± 0.030	2.68	0.886 ± 0.046	29.1 ± 3.3	258^{+86}_{-46}
76NZ6a-1	layer B	0.896 ± 0.031	2.27	0.851 ± 0.073	50.3 ± 26.5	228^{+127}_{-54}
76NZ6a-2	layer B	0.887 ± 0.020	2.26	0.834 ± 0.032	52.5	214^{+33}_{-25}
76NZ6a-3	layer B	0.929 ± 0.020	1.87	—	—	—
76NZ6a-4	layer B	0.890 ± 0.018	2.75	0.804 ± 0.062	>1,000	191^{+5}_{-36}
	Average, layer B	0.900 ± 0.019	2.37			211 ± 19
76NZ6d-3	layer D	1.076 ± 0.030	0.97	—*	—	—
76NZ6d-4	layer D	1.125 ± 0.064	1.34	0.584 ± 0.040	>1,000	85.2 ± 10.0
76NZ1	layer D	1.060 ± 0.030	2.12	0.496 ± 0.023	>1,000	74 ± 5
Ein Aqev						
76NZ8	Fossil spring deposit	0.963 ± 0.032	2.30	0.110 ± 0.006	17.1	$11.8 \pm 0.9^\dagger$

Errors are 1σ , based on Poisson statistics for α -counting.

* No thorium recovered.

† Corrected for initial $^{230}\text{Th}/^{232}\text{Th} = 1.5$ at $t = 0$.

‡ See Fig. 2 for location of layers A to E.

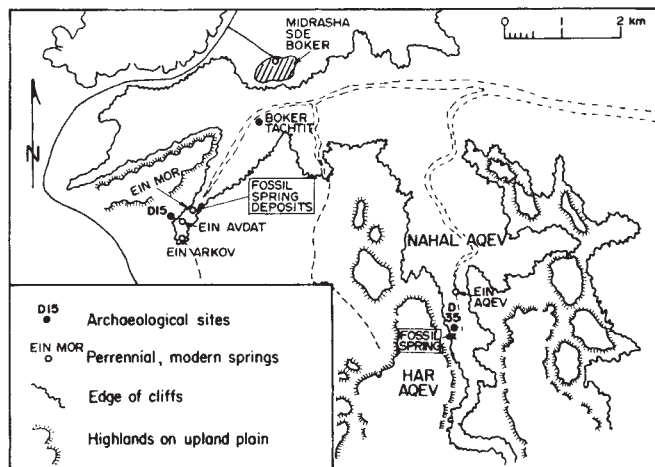


Fig. 1 Map of Nahal Zin showing locations of dated sites. Nahal Mor is the canyon in which Ein Mor and Ein Avdat are located.

also closely resemble the lithic technology of site Rosh Ein Mor (D 15), situated on the canyon rim above Nahal Mor.

The lowest layer of the travertine mound (Fig. 2A) was deposited $258,000 \pm 66,000$ yr BP, whereas three dates from layer B give an average age of $211,000 \pm 19,000$ yr BP. Layer C is undated. From layer D, two samples were obtained, one with an apparent age of $85,000 \pm 10,000$ yr BP (including a small correction for common ^{230}Th) and another (76NZ1) giving an age of $74,000 \pm 5,000$ yr BP. Unfortunately, both the artefact-bearing layer and the top layer (E) are too heavily contaminated with limestone fragments to permit dating. The artefact-bearing layer, which seems stratigraphically to be either of the same age or slightly younger than layer D, was therefore deposited at about $80,000 \pm 10,000$ yr BP. We infer that this was the time of occupation of site D 35, the location of which was presumably determined by the proximity of the spring¹². The site may have been abandoned when flow from the springs ceased.

Further downstream in Nahal Aqev, the active spring of Ein Aqev is now depositing travertine on the steep walls of a box canyon. Older deposits a few metres downstream on the west side of the canyon mark earlier stages in the headward recession of the canyon. A sample from the surface of these deposits (76NZ8) shows that the spring was active $12,200 \pm 700$ yr BP; from the thickness of the deposits we infer that deposition started a few thousand years before that date, $\sim 15,000$ yr BP. This was close to the time of occupation of the nearby Upper Palaeolithic site D 31 (Ein Aqev), ^{14}C -dated at from 18,000 to 17,000 yr BP¹³.

These results, as well as providing the first absolute date for the early Levantine Mousterian culture, demonstrate the potential of $^{230}\text{Th}/^{234}\text{U}$ dating of travertine in establishing an absolute time scale in archaeology.

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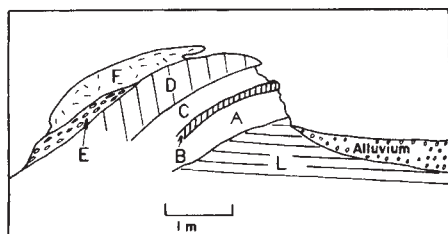


Fig. 2 Cross-section of fossil spring mound in Nahal Aqev. Layers A to D: travertine; layer E: colluvium containing Levallois flakes; layer F: travertine containing abundant limestone clasts; L: limestone bedrock.

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Phylogeny and palaeobiogeography of hadrosaurian dinosaurs

MOST geologists agree on the placement of the continents at the end of the Cretaceous. Palaeobiologists, however, hold divergent opinions^{1,2}. Some workers connect South America to Africa, some connect it only to North America, while others isolate South America totally. New evidence from vertebrate palaeontology suggest that a substantial land connection between North and South America existed 85–100 Myr ago which facilitated the passage of many terrestrial vertebrates between the continents, including large herbivorous dinosaurs. Specimens of hadrosaurian dinosaurs, collected in the first half of the present century, have now been used to reanalyse not only the palaeobiogeography but also the classification and phylogeny of this group. I report here the first solid evidence of an extensive land connection for large terrestrial vertebrates of this sort between North and South America at the end of the Cretaceous.

Hadrosaurs were the most diverse and abundant late Cretaceous, terrestrial vertebrate herbivores. In most cases they seem to have comprised up to 75% of the biomass in local faunas³, according to recovered fossil samples. Their remains can be found in every Laurasian continent and also in South America. Because most specimens are represented by post-crania alone, and most workers believe that hadrosaur post-crania are not diagnostic, it has been almost impossible to map the distribution of sub-familial taxa. It is now possible, especially with pelvic elements, to identify a genus on the basis of a single element⁴. With this new information, previously named taxa can be rediagnosed, isolated elements from all over the world can be identified, and Cretaceous hadrosaurian palaeobiogeography can be reanalysed.

An accurate, useful phylogeny must include all valid taxa from all geological ages and all localities. Previous hadrosaur phylogenies have been based on North American data only^{5,6}. My new phylogeny (Fig. 1) includes all forms at the generic level and recognises two subfamilies, the Hadrosaurinae and the

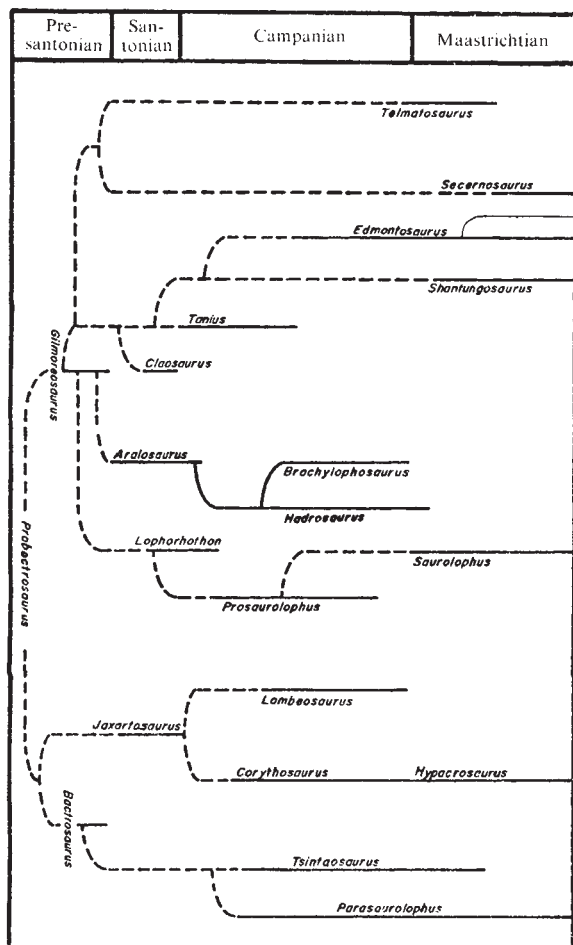


Fig. 1 Phylogeny of the hadrosaurs. All valid genera from all the continents are listed. 'A' coming off the *Edmontosaurus* lineage represents *Anatosaurus copei* only. This form will be redescribed as a new genus elsewhere. All other species of *Anatosaurus* are considered junior synonyms of *Edmontosaurus regalis* and *E. edmontoni*. There is a distinct possibility that these two species may be male and female respectively. *Bactrosaurus*, *Jaxartosaurus* and their descendants are lambeosaurines—all other genera are hadrosaurines. *Tsintaosaurus* is transferred from the hadrosaurines to the lambeosaurines based on postcranial studies and the presence of a hollow crest. I follow Dodson¹³ in considering 'procheneosaurs' as juvenile forms of *Corythosaurus* and *Lambeosaurus*. *Cheneosaurus* is certainly a juvenile *Hypacrosaurus*. *Brachylophosaurus* is transferred from the *Saurolophus* lineage to the *Hadrosaurus* (*Kritosaurus*) lineage based on postcranial and cranial evidence.

Lambeosaurinae. The Hadrosaurinae are characterised by: relatively low sacral neural spines with a length/width ratio less than 4.5; ventrally grooved sacral centra; long gracile limb elements, non-footed ischium; prepubis with a long thin neck; ilium length/height ratio averaging 4.4; convoluted or slightly expanded pre-maxilla; long straight mandible; solid narial crests (where present). The Lambeosaurinae are characterised by: relatively high sacral neural spines with a length/width ratio greater than 4.5; ventrally ridged sacral centra; shorter and more robust limb elements; footed ischium; prepubis has a short, thick neck; ilium length/height ratio averages 5.4; greatly expanded premaxilla; shorter and anteriorly decurved mandible; hollow narial crests present. Most, if not all, genera in both subfamilies are monospecific. Evidence based on postcranial studies⁴ suggests that the *Saurolophinae* should be included in the Hadrosaurinae.

The three pivotal genera in this report are *Secernosaurus koernerii* (new genus, new species) from Argentina, and *Gilmoreaosaurus mongoliensis* (new genus, new combination) and *Bactrosaurus johnsoni*¹⁷ both from Mongolia. All three

genera display a primitive grade of organisation reminiscent of their iguanodont ancestry. *Secernosaurus* is the second most primitive hadrosaur known. It is only the second hadrosaur reported from Gondwanaland⁸ and the first to be of diagnostic value (the first was by R. Casamiquela). The unique features of its ilium justify a new genus:

Class, Archosauria
Order, Ornithischia
Family, Hadrosauridae
Subfamily, Hadrosaurinae
Secernosaurus (new genus)

Holotype: FMNH P13423, two ilia, prepubis, scapula, fibula, caudals and partial braincase. Collected in 1923 by J. B. Abbott for the Field Museum of Natural History in Chicago.

Horizon: San Jorge Formation, Rio Chico, Patagonia, Argentina.

Age: Upper Cretaceous.

Etymology: generic name from the Latin *secerno* which means to sever or divide, referring to its non-Laurasian origin.

Diagnosis: postacetabular process of ilium greatly deflected dorsomedially and elongate, unlike any hadrosaur or iguanodont. Preacetabular process of ilium deflected ventrally at an angle greater than in *Hadrosaurus* (Fig. 2). (I follow Baird and Horner⁹ in recognising *Hadrosaurus* as the senior synonym of *Kritosaurus*.) Antitrochanter relatively smaller than in any hadrosaur of the same size.

Secernosaurus koernerii (new species)

Diagnosis: same as for genus.

Etymology: specific name after Dr Harold E. Koerner, professor emeritus, University of Colorado.

Gilmoreaosaurus is the first representative of the Hadrosaurinae in Asia. It was originally described as a new species of the genus *Mandschurosaurus*⁷ but this genus was proved to be a *nomen dubium*⁴. The species, however, contains many features that are partly iguanodont and partly hadrosaurine, hence it is appropriate to recognise this form as a valid genus:

Gilmoreaosaurus (*Mandschurosaurus*) *mongoliensis*
(new genus, new combination)

Holotype: redesignated American Museum of Natural History 6551.

Horizon: Iren Debasu Formation, Cenomanian?

Diagnosis: same as original description for species⁷.

Etymology: after Charles W. Gilmore, the original author.

Bactrosaurus, the first known lambeosaurine, occurs with *Gilmoreaosaurus* in the same formation. The two subfamilies (Lambeosaurinae and Hadrosaurinae) are already separate at this time, which would put the origin of the family some time in the Middle Cretaceous.

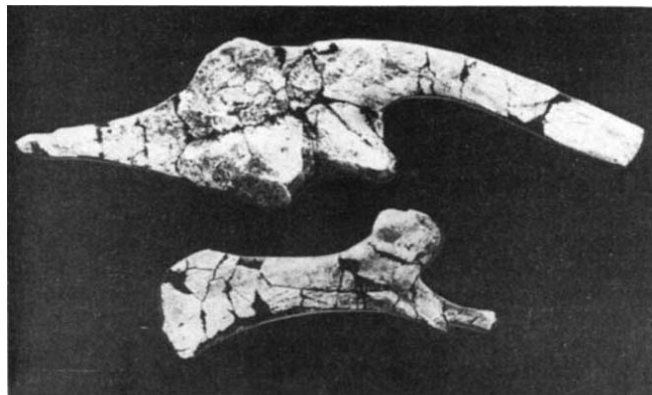


Fig. 2 *Secernosaurus koernerii* (new genus and species). Holotype FMNH P13423. Right ilium and left prepubis in lateral view. Length of ilium between perpendiculars is 533 mm.



Fig. 3 Palaeogeographic reconstruction of the continents during the Turonian Transgression. Based on Tedford² and Smith and Briden¹¹.

Hadrosaurs^{7,10} are considered to have originated in Asia where *Proactosaurus*, an iguanodont and probable ancestor, has been found. The predominance of primitive hadrosaurs and advanced iguanodonts in Laurasia does not necessarily imply that the family originated there. The fossil record only indicates that by the Mid Cretaceous, hadrosaurs were present in both Laurasia and Gondwanaland, and that they had differentiated into two subfamilies. Dispersal between South America and Laurasia could have been by way of Africa or North America (Fig. 3). Most workers^{2,11} believe that Africa was isolated from South America sufficiently early so as not to have been involved in the Upper Cretaceous dispersal of marsupials, so that dispersal must have occurred by way of North America. The two possible links with South America were through Panama or the Caribbean arc. Both hadrosaurs and ceratopsians¹² are found in South America and so the route used must have been a major land connection at some time because both groups consist of large terrestrial vertebrates not subject to waif dispersal or rafting. Dispersal is not considered to have occurred after the Late Cretaceous transgression, because Santonian hadrosaurs were already advanced (derived) beyond the *Secernosaurus* grade of evolution.

It is possible that the presence of hadrosaurs in Laurasia and Gondwanaland represents vicariance rather than dispersal; if so, hadrosaurs would be expected to occur on most continents. Their absence in India and Australia, where Upper Cretaceous deposits with vertebrates are known, indicates otherwise. This, however, is negative evidence and disprovable. Until hadrosaurs are found in India and Australia, the dispersal model is to be favoured.

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Kanamycin-resistant *Scenedesmus obliquus* showing simultaneous resistance to other antibiotics and polychlorinated biphenyls

WE have been studying the effects of polychlorinated biphenyls (PCBs) and DDT on several planktonic algae, and report here that we have isolated a kanamycin-resistant strain of *Scenedesmus obliquus* which shows simultaneous resistance to other antibiotics, PCBs and Cetavlon (ICI), a quaternary ammonium compound with algicidal properties². This novel observation suggests a possible genetic control of sensitivity to PCBs in *Scenedesmus*.

S. obliquus (UTEX 2015) was one of the test organisms and was obtained as an axenic culture. This strain was grown mixotrophically in liquid TAP (Tris-acetate-phosphate)⁴ and when phototrophic growth was being tested acetate was omitted from the medium, which was then a minimal TAP (M-TAP). *S. obliquus* was able to grow on M-TAP in light, thus confirming it to be a phototrophic strain. Agar plates were prepared by adding 1.5% of agar (Difco) to the liquid medium. Required concentrations of antibiotics were filter sterilised and added just before the plates were poured. To test for sensitivity or resistance to antibiotics, 0.1 ml of a *S. obliquus* culture containing 1×10^7 cells ml^{-1} was spread on TAP- and M-TAP-containing antibiotic plates. The plates were incubated for a week in a growth cabinet at 22 °C with continuous illumination. Growth was determined visually.

The control plates without antibiotics showed a heavy growth in the form of a continuous green lawn; there was no comparable growth on antibiotic-containing plates. Thus, we concluded that *S. obliquus* was sensitive to kanamycin ($100 \mu\text{g ml}^{-1}$), chloramphenicol ($50 \mu\text{g ml}^{-1}$), streptomycin ($300 \mu\text{g ml}^{-1}$) and tetracycline ($50 \mu\text{g ml}^{-1}$). However, when these plates were held at 22 °C for 1 month, we found 7-8 discrete, actively growing colonies on each of the kanamycin-treated plates only. These

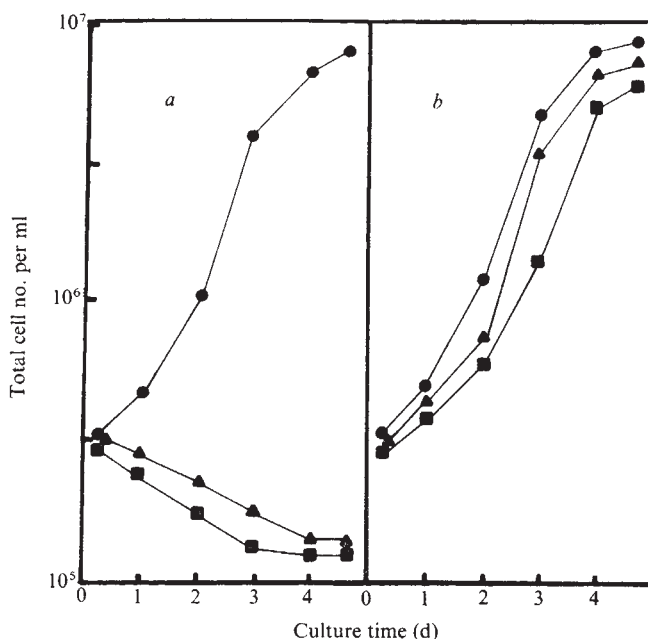


Fig. 1 Effects of PCBs (Aroclor 1242) and Cetavlon on the growth of *S. obliquus* (a) and kanamycin-resistant *S. obliquus* (b). Cells were grown in liquid TAP and total cell number represents viable cell counts within a 90% confidence level. ●, control; ▲, 5 p.p.m. Aroclor 1242; ■, 5 p.p.m. Cetavlon.

colonies were isolated and named 2015-kr (kanamycin resistant) cells of *S. obliquus*, and cells from each colony were tested separately on TAP plates containing various antibiotics. The progeny of each colony showed similar results and so were treated as a single resistant strain for further experiments. When these presumptive mutants were tested on kanamycin, streptomycin, chloramphenicol and tetracycline plates, they all showed simultaneous resistance on TAP plates. The property of simultaneous resistance had become a permanent feature; this was confirmed by repeated tests on antibiotic-containing plates. Surprisingly, 2015-kr cells failed to grow when plated on TAP media without acetate (M-TAP) supplied with the antibiotics, although the original strain could grow equally well on M-TAP or TAP.

Examination under the optical microscope showed these cells (2015-kr) to be smaller and more round than the parental strain, and lacking coenobia, characteristic of *Scenedesmus*. However, to prove that these were a true derivative of *Scenedesmus* and not a contaminant of another alga, we grew this strain on several different media including some which were unfavourable for active growth of *S. obliquus*. In one such medium, proteose agar, the resistant cells did not show active growth but did produce typical *Scenedesmus* morphology³. The identity of these cells was then independently confirmed as *Scenedesmus*.

Previously, *Scenedesmus* (2015) was found to be sensitive to PCB at 0.25 p.p.m. and to an algicide, Cetavlon, at the 1 p.p.m. level. When the kanamycin-resistant variety was tested on these chemicals it was found to be resistant above the 5 p.p.m. level, a concentration lethal for the parent strain (Fig. 1).

The fact that 2015-kr cannot grow on the M-TAP containing antibiotics suggests that the genes affected here are possibly chloroplastic. However, both the parental strain and 2015-kr could grow on M-TAP without antibiotics; this indicates that *S. obliquus* was phototrophic whereas 2015-kr behaved as an obligate heterotroph in the presence of antibiotics. Extensive work on the inheritance of antibiotic resistance involving chloroplastic genes has been carried out in *Chlamydomonas reinhardtii*^{1,9-12}, where it was shown that many of the mutations conferring antibiotic resistance were present in clusters in chloroplastic DNA. Streptomycin has been used as a selective agent to isolate resistant strains to other antibiotics in *C. reinhardtii* and these mutations were also mapped as chloroplast mutations¹. However, *C. reinhardtii* possesses a sexual cycle which enables easy differentiation between nuclear and cytoplasmic genes, whereas *Scenedesmus* reproduces asexually and so it is not possible to carry out genetic crosses. Although there is one report of sexuality⁸ in *Scenedesmus*, it has not yet been confirmed.

Our previous studies on *Chlorella* and *Spirodella*⁵⁻⁷ in relation to their sensitivity to PCBs showed that photosynthesis was inhibited by these chemicals. Sensitivity to PCBs could be decreased by addition of a supplementary carbon source to the medium which allowed the cells to by-pass the inhibitory effects of PCBs (in preparation). We believe this to be the first report of an alga, previously sensitive to PCBs and several antibiotics known to affect chloroplast ribosome synthesis, which has acquired simultaneous resistance to all these chemicals. This suggests that, for *Scenedesmus* at least, responses to environmental pollutants such as PCBs can be controlled by specific genes similar to those conferring antibiotic sensitivity. These genes seem to be located in the chloroplast, but the possibility of their being nuclear genes concerned with the function of cytoplasmic organelles is not ruled out.

More recently, we have been able to repeat the isolation of kanamycin-resistant *Scenedesmus* cells; *S. obliquus* will apparently produce approximately one resistant cell per 5×10^5 cells, but this mutation only occurs on kanamycin plates when grown over a period of 1 month. These new isolates are under further investigation.

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Ciliate protozoans as links in freshwater planktonic food chains

THE potential importance of bacteria in freshwater planktonic food webs is recognised¹, but the mechanisms of their incorporation are not fully known. Most planktonic bacteria are not attached to particles and are less than 1 μm (refs 2-4) in size—a range inefficiently filtered by crustacean zooplankton⁵. *Daphnia* and *Diaptomus* feed at lower rates on free living bacteria than on algae^{6,7}. Bacterial food sources have been used with varying degrees of success to culture zooplankton⁸⁻¹¹. These variations may be due to the unnatural range of concentrations used and the uncontrolled effects of different culture media, laboratory strains and the degree of accessibility to the zooplankton (because of cell clumping and sedimentation). Ciliate protozoan microzooplankton may be important intermediaries which transform ultrafine organic matter, including bacteria, into a particle size range readily available to crustacean zooplankton. Here we present evidence that ciliates are cropped by crustacean zooplankton in the natural environment. We have also measured feeding, filtering, and assimilation rates of *Daphnia magna* on both a large and small ciliate in the laboratory and have observed feeding behaviour directly. Dissolved organic carbon, bacteria, ciliate protozoans and crustacean zooplankton may represent a significant pathway of carbon flow in the freshwater plankton.

Ciliate protozoa ingest bacteria in nature¹² and grow on them as a sole food source in the laboratory¹³. Furthermore, field studies indicate that planktonic ciliates are actively preyed on in nature. Manipulations of crustacean zooplankton densities in enclosures of lake water¹⁴ reveal that ciliates, as well as algae, are reduced in abundance when crustacean zooplankton densities increase (Table 1). In these field studies, remains of ciliates were found in the feeding appendages and gut contents of the copepod *Cyclops scutifer*. Other microzooplankton, the rotifers, were unaffected by manipulations of crustacea, indicating that they are not major links in this system. Similar reductions in protozoan numbers in the presence of marine copepods occurred in laboratory studies¹⁵.

Feeding studies of *Daphnia magna* were made using the large ciliate *Paramecium caudatum* ($70 \times 200 \mu\text{m}$; volume $6.15 \times 10^6 \mu\text{m}^3$) and the small ciliate *Cyclidium glaucoma* ($20 \times 30 \mu\text{m}$; volume $6.54 \times 10^4 \mu\text{m}^3$). Protozoans were raised on suspensions of *Enterobacter aerogenes*. Bacteria were grown for 24 h at 38 °C in defined medium, and resuspended in Cerophyll to a final concentration of 1×10^6 cells ml^{-1} . Protozoans were saturation labelled by incubating in this suspension for 7 d at 24 °C. They were separated from the bacteria by geotaxis¹⁶ and

Table 1 Effect of manipulations of crustacean zooplankton on algae and microzooplankton

	Levels of crustacean abundance*			χ^2	P
	Absent	Natural	Enriched		
Ciliates	35.0	25.0	12.0	22.167	<0.001
Total algae	35.0	24.0	13.0	20.167	<0.001
Rotifers†	24.5	33.5	18.0	1.792	NS

**Daphnia galeata mendotae*, *Diaptomus minutus*, *Cyclops scutifer*, and *Epishura lacustris* were removed by filtration or enriched 8-fold in 0.5 m³ enclosures of lake water on 12 occasions throughout the year¹⁴. Whole water was collected from the enclosures after 4 d, fixed with Lugol's iodide preservative and counted with an inverted microscope. Because of seasonal variations in abundance, data are reported as sums of ranks. Actual ciliate counts from natural epilimnetic lake water ranged from 0.3 ml⁻¹ to 9.0 ml⁻¹. Ranks were summed for each level over the 12 dates and used to compute a Friedman χ^2 with a critical value of 6.167 ($P < 0.05$). NS, not significant.

†*Keratella*, *Kellicottia* and *Conochilus*.

resuspended in filtered (0.45 μ m Millipore), autoclaved aquarium water in which the experimental *D. magna* were raised. Protozoans were sized, counted in a Sedgewick-Rafter cell and diluted to a final concentration of 135 individuals ml⁻¹ (0.985 μ g dry weight ml⁻¹) for *P. caudatum* and 2.55×10^3 individuals ml⁻¹ (0.823 μ g dry weight ml⁻¹) for *C. glaucoma*. Both concentrations are above the incipient limiting concentration for *D. magna* and thus feeding rates were maximised.

Observations of *D. magna* feeding on individual *P. caudatum* were made using an inverted microscope (Zeiss) with 10 $\times$ eyepieces and a 63-mm Luminar objective permitting a working distance of 6.0 cm and a 2.0-mm depth of field. Live *D. magna* were suspended in a large volume feeding chamber (5 $\times$ 5 $\times$ 5 cm) by attaching the head shield to a fine glass rod tipped with silicone grease. This is an improvement over previous methods of observing compressed *Daphnia*, as it allows free circulation of feeding currents and unobstructed movements of the abdomen, swimming appendages and carapace.

The bacterium *Enterobacter aerogenes* assimilated the ¹⁴C-labelled glucose and served as a food source for the protozoans which incorporated the label. *D. magna*, in turn, assimilated this carbon from the protozoans it ingested. Filtering rates of *D. magna* fed on either protozoan showed a positive linear relationship to body size (Fig. 1). *D. magna* ingestion, filtering, and assimilation rates were higher when fed the smaller protozoan, *C. glaucoma*, which is in the size range of many algae, than when fed the large *P. caudatum* (Table 2). Ingestion and filtering rates on both protozoa were lower than rates measured in similar conditions on algae, although the assimilation efficiencies were comparable¹⁷.

Table 2 Mean ingestion rates, assimilation rates, and assimilation efficiencies for *Daphnia magna* fed on *Paramecium caudatum* and *Cyclidium glaucoma*

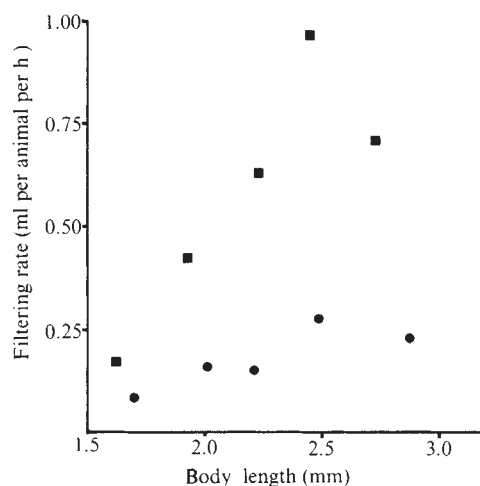
Food organism	Ingestion rate*		Assimilation rate*		Assimilation efficiency
	$\bar{x} \pm \text{s.e.m.}$	N	$\bar{x} \pm \text{s.e.m.}$	N	
<i>P. caudatum</i>	0.1785 $\pm$ 0.0335	5	0.1166 $\pm$ 0.0103	4	65%
<i>C. glaucoma</i>	0.4793 $\pm$ 0.0110	5	0.3161 $\pm$ 0.0303	4	66%

Ingestion and assimilation rates were measured by adding 25–35 *D. magna* to replicate 300 ml BOD bottles containing protozoan suspensions and measuring radioactive uptake in 15 min and incorporation during 1 h (ref. 17). *D. magna* of comparable body sizes were used to measure ingestion rates and assimilation rates for the two species of protozoa. Assimilation efficiencies are calculated as the per cent of the material ingested that is assimilated.

*Ingestion rates ($t = 2.608$, $P < 0.025$) and assimilation rates ($t = 6.232$, $P < 0.001$) were significantly different for the two protozoa.

Rates are expressed as μ g dry weight of food per animal per hour. Abbreviations: $\bar{x}$, mean; s.e.m. standard error; N, No. of replicates.

Observations of *Daphnia magna* feeding on *P. caudatum* indicated that only some of the protozoans that entered the filtering current were brought inside the carapace and into the food groove. These were broken up by the movement of the thoracic appendages, labrum, and mandibles and ingested with some loss of material. This slovenly feeding in *Daphnia* is analogous to loss of food material in the mouth parts of other planktonic crustacea¹⁸. This confirms the interpretation of observations made of animals feeding on large or elaborately shaped algal cells and colonies¹⁹; food particles, with dimensions larger than the maximum size of rigid spherical particle (38 μ m for *D. magna*)²⁰ that can be ingested whole, can be manipulated and ingested but with reduced efficiency. Measured filtering rates of these particles will be less than the actual rate at which water passes through the filtering apparatus because of rejection of particles that clog the filtration apparatus and loss of material broken up during filtration. The upper and lower limits to the particle size ranges available to *Daphnia* are thus not absolute.

**Fig. 1** Filtering rates of *Daphnia magna* on the small ciliate (20 $\times$ 30 μ m, *Cyclidium glaucoma* (■), and the large ciliate (70 $\times$ 200 μ m), *Paramecium caudatum* (●).

Rather, there is a decrease in handling efficiency as particles move away from an optimal size range. Optima for different age classes and species may be the mechanisms by which zooplankton divide up food resources. Behavioural changes may allow individuals to shift optima to accommodate different food particle spectra.

Studies of ciliates feeding on bacteria suggest that a minimum concentration of approximately 10^6 bacteria ml⁻¹ is necessary for growth and reproduction of protozoans^{21,22}. Nominal concentrations of bacteria in lakes range from 10^5 to 10^6 ml⁻¹ (ref. 2). During the declining phase of algal blooms, however, bacterial production is elevated and high concentrations of ciliates (10^2 ml⁻¹) have been observed²³. Protozoans may also be concentrated in zones near the metalimnetic boundaries, at chemoclines, and above the sediments²⁴. If these organisms are cropped by larger zooplankton, as our field and laboratory experiments suggest, then they represent, at least temporally, an important mechanism by which DOM, bacteria, and ultrafine detritus enter planktonic food chains.

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Low levels of fixed nitrogen required for isolation of free-living N₂-fixing organisms from rice roots

ISOLATION and counting of N₂-fixing bacteria from the natural environment have until now been conducted on selective N-free media. It is likely that some N₂-fixing bacteria are overlooked by using this procedure. We now report that most of the bacteria present in rice roots are N₂-fixers, but that they require a supply of mineral or organic N for growth. In the presence of organic nitrogen, nitrogenase activity is detected in these organisms. The requirement for organic nitrogen for the detection of nitrogenase is also true for N₂-fixing cultures of free-living rhizobia^{1–3}.

Rice plants (IR26) were dug out from an unfertilised wetland rice field and the roots were washed to remove soil. The washed roots were cut into segments and shaken vigorously with glass beads to remove rhizoplane bacteria. After several washings the root fragments were macerated to provide a suspension of 'inner rhizoplane' bacteria. The lower portion of the stem was also blended to make a suspension of bacteria living in or on the stem. Suspensions were made in sterile tap water and diluted without exclusion of oxygen. Diluted suspensions were spread on tryptic soy agar (0.1% Difco tryptic soy broth and 1.5% Noble agar), a medium used for counting aerobic heterotrophs⁴. The population of heterotrophic aerobic bacteria per g fresh weight sample was 2.3×10^7 organisms for rhizosphere soil, 1.2×10^7 for stem, 1.5×10^7 for rhizoplane, and 1.1×10^8 for inner rhizoplane.

Colonies from each fraction were selected and tested for nitrogenase activity using the acetylene reduction assay. Acetylene reduction activity (ARA) was measured for a 24-h period after 3 days growth at 35 °C on semi-solid glucose yeast extract medium (in g l⁻¹: 5.0 glucose, 0.1 Difco yeast extract, 0.5 K₂HPO₄, 0.04 FeSO₄ · 7H₂O, 0.2 MgSO₄ · 7H₂O, 0.0059 NaMoO₄ · 2H₂O, 0.2 CaCl₂ · 2H₂O; in mg l⁻¹: 0.15 H₃BO₃, 0.11 ZnSO₄ · 7H₂O, 0.07 CoSO₄ · 7H₂O, 0.005 CuSO₄ · 7H₂O, 0.004 MnCl₂ · 4H₂O, pH 7.0, and Difco Noble agar 1.75 g l⁻¹). The percentage of bacterial isolates proving nitrogenase positive (more than 6 nmol C₂H₄ per tube) was 81% (95/117) for inner rhizoplane bacteria, 76% (19/25) for rhizoplane bacteria, 37.5% (9/24) for bacteria from the stem, and 2.4% (1/41) for rhizosphere soil bacteria.

Because of the high percentage of inner rhizoplane isolates showing nitrogenase activity, the nutritional requirement of bacteria of the most predominant colony type (about 90% of nitrogenase positive isolates) was studied further. None of the isolates could grow on nitrogen-free basal medium (glucose +

mineral salts). The majority (31 of 40) grew on basal + ammonium medium (NH₄Cl 61 mg l⁻¹), but the growth was much poorer than on basal medium supplemented with casamino acids (vitamin-free, Nutritional Biochemicals) or yeast extract (0.1 g l⁻¹).

For isolates that grew best on basal medium plus casamino acids, nitrogenase activities were measured after 3 days of growth on various semi-solid media (Table 1). Activities were high in the presence of casamino acids but a vitamin mixture only slightly stimulated activity. No activity was detected in N-free medium or ammonium-supplemented medium. Each amino acid was tested individually and all could support growth and nitrogenase activity.

Table 1 Acetylene-reducing activity of inner rhizoplane isolates in semi-solid media

Medium	C ₂ H ₄ produced (nmol per tube per day)
Expt 1 (28 isolates tested)	
Basal (glucose + mineral salts)	0
Basal + yeast extract (0.1 g l ⁻¹)	97 ± 11
Basal + NH ₄ Cl (16 mg N l ⁻¹)	0
Basal + casamino acid (0.1 g l ⁻¹)	96 ± 18
Basal + glutamic acid (0.1 g l ⁻¹)	39 ± 8
Expt 2 (29 isolates tested)	
Basal + yeast extract	219 ± 17
Basal + casamino acid	144 ± 13
Basal + glutamic acid	120 ± 22
Basal + yeast extract + succinic acid (10 mM)	202 ± 14
Expt 3 (29 isolates tested)	
Basal + casamino acid	222 ± 7
Basal + vitamin mixture*	58 ± 4
Basal + vitamin mixture + casamino acid	251 ± 13

*Vitamin mixture: vitamin B12, 0.015 μM; *p*-aminobenzoic acid, nicotinic acid, biotin, riboflavin, pyridoxine, folic acid, and Ca-pantothenate, all 0.8 μM.

The extent of inhibition of nitrogenase activity in the presence of oxygen was determined. When initial *p*O₂ was 0.01 atm, nitrogenase activity was maximal while at *p*O₂ 0.2 atm and 0.05 atm, nitrogenase activity was not detected. In anaerobic (*p*O₂ < 0.001) culture, slight activity of nitrogenase was detected. Like other aerobic N₂-fixing bacteria, such as *Azospirillum*, these bacteria require microaerophilic conditions for the expression of nitrogenase.

To confirm N₂-fixing activity a gas mixture composed of 20% ¹⁵N₂ (98 atom % excess), 1% O₂, 79% Ar was introduced into 4-day static bacterial cultures on basal medium plus glutamate (0.1 g l⁻¹) and grown for the following 24 h at 35 °C. The two isolates used for the analysis incorporated 0.18–0.25 atom % excess into cellular nitrogen. *Azospirillum lipoferum* (SpBr17), which was used as a positive check strain, showed a higher rate of incorporation (1.16 atom % excess). Excretion of fixed nitrogen into the medium was negligible in all cases.

Nitrogenase-positive isolates from the inner rhizoplane were all small rods (0.5 × 2.6 μm), Gram-negative, catalase and oxidase positive, lacked cellular motility, and produced acid from lactose, sucrose, and glucose without gas formation and reduced nitrate. Thus these isolates resemble *Achromobacter*^{5,6}. The only known N₂-fixing isolate of this genus was reclassified as *Klebsiella pneumoniae*⁷.

A possible explanation for the inability of the isolates to grow on nitrogen-free media is that the nitrogenase activity (as measured by ¹⁵N experiments) is too low. A low oxygen concentration produced in the presence of greater cell mass might be more favourable for nitrogenase production, however. The

oxygen factor is another possible explanation of the growth characteristics. Free-living N_2 -fixing rhizobia⁸ are unable to grow on N-free media because they cannot assimilate fixed nitrogen: this is probably not the case for our root isolates as excretion of fixed nitrogen into the medium was negligible. We have not yet studied the role of amino acids in nitrogenase induction.

The use of nitrogen-containing media for the isolation of nitrogenase-positive bacteria could reveal a much higher incidence of nitrogenase genes in prokaryotes than was previously suspected.

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Postnatal cerebellar cells of *staggerer* mutant mice express immature components on their surface

THE histogenesis of the central nervous system is controlled, in part, by a defined sequence of cell-surface interactions^{1,2}. Cell-surface carbohydrates are prominent constituents of the external faces of plasma membranes^{3–5} and are thought to be important to biological recognition^{6,7}. Recently, it has also been shown that some cell-surface carbohydrates change during the first 2 d after birth, an important period in development of the mouse cerebellum^{8,9}. Certain neurological mutations have been shown to express defects in positioning of particular cell types at various stages during cerebellar histogenesis^{2,11–13}. One of the most interesting of these is the *staggerer* (*sg*) mutant¹⁴. In this disorder granule cells degenerate after they have migrated into position in the granular layer. Before this expression of the disorder, the sites of synapse formation between the parallel fibre axons of granule cells and the dendrites of Purkinje cell neurones fail to form; in fact, even the tertiary branchlet spines, the potential postsynaptic sites, do not form^{15–19}. Even at birth, before obvious expression of the Purkinje cell defect, external granule cells of *sg* exhibit a reduced proliferative rate which results in a reduced number of external granule cells²⁰.

In this report, antibodies specifically directed against microbial polysaccharides have been applied as carbohydrate probes to developing cerebellar cell populations *in vitro*. Anti-meningococcus type-B polysaccharide antibodies (anti-B) (antigenic determinant neuraminic acid) preferentially react with pre- and neonatal cerebellar cells of C57BL/6J mice, but do not react with 7-d-old (P7) cells. Anti- α -1,6-mannan antibodies, on the other hand, react with both prenatal and postnatal cerebellar cells. Comparable results have been obtained with lectins as surface carbohydrate probes¹⁰. Agglutination of cerebellar cells with concanavalin A and wheatgerm agglutinin decreased from

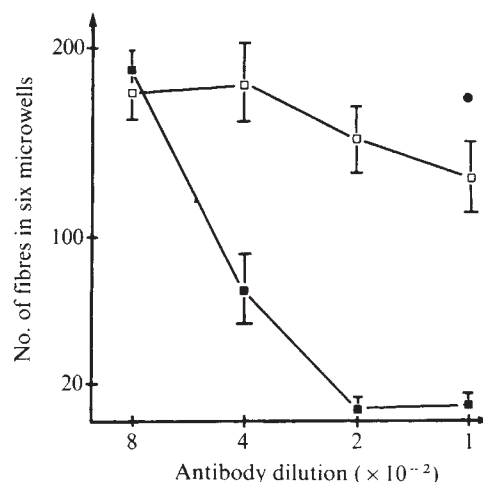


Fig. 1 Inhibition of fibre formation in microwell cultures of 7-d-old *sg/sg* cerebellum in the presence of various dilutions of anti-meningococcus type-B antibodies (anti-sialic acid) (■). The inhibition was overcome when antibodies were incubated overnight with 10 μ g of meningococcus type-B polysaccharide before adding the antigen-antibody complex to the culture (□). Normal rabbit serum did not inhibit fibre formation (●). The numbers plotted represent the mean of three experiments of the total number of fibres in six microwells.

embryonic day 13 (E13) to postnatal day 7 (P7). Using anti-B and anti- α -1,6-mannan antibodies to determine the distribution of their respective antigens on the surface of $+/+$ and *sg/sg* cerebellar cells *in vitro*, we found that P7 *sg/sg* cell cultures express immature cell-surface carbohydrates.

The present work is one step in a study to determine whether the defect in some of these neurological mutants might be in cell-surface constituents and whether their alterations might affect the sequence of cell-cell interactions required to generate functional connections in the developing cerebellum.

A microwell tissue culture system, in which cells show certain behaviours reminiscent of *in vivo* cerebellar development²¹, was used to determine anti-carbohydrate antibody reactivity. This system allows the assay of differential cell assembly, migration and fibre outgrowth. The large number of very reproducible

Table 1 Fibre outgrowth in microwell cultures of 7-d-old *sg/sg* and $+/+$ cerebellum in the presence of anti-carbohydrate antibodies

Expt	Antibody	Dilution	No. of fibres in 6 microwells			
			$+/+$	Inhibition %	<i>sg/sg</i>	Inhibition %
1	Rabbit serum	1:50	180		193	
2			178		169	
3			222		176	
1	Anti-B	1:200	162	10	16	92
2			148	17	12	93
3			195	12	38	78
1	Anti- α -1,6-mannan	1:300	14	92	129	33
2			2	99	118	30
3			8	96	108	39

A single-cell suspension (1×10^7 cells ml^{-1}) of 7-d-old $+/+$ and *sg/sg* cerebellum, respectively, were plated at 5 μ l per microwell, and 5 μ l of antibody dilution in medium then added. Fibre formation was scored in six microwells after 2 d *in vitro* using a phase contrast light microscope. The variation among cultures was determined as $\pm 20\%$. The data represent the total number of fibres in six microwells obtained in three different experiments. The number of fibres obtained in cultures containing rabbit serum were used as standard for 100% fibre formation per experiment.

cultures obtained from each cerebellum provides a statistically suitable assay system for study of the functional effect of reagents that bind to specific cell-surface components. The number of fibre cables formed between cell aggregates was determined in the present study as a quantitative assay for antibody reactivity.

sg/sg Mutant mice were obtained on an inbred background (C57BL; $^{+}dse/sg^{++}$) as bred in our own colony. P7 cerebella of *sg/sg* and $+/+$ (*dse/dse*) were removed and dissociated into single cells as described elsewhere²¹. Single-cell suspensions contained $2-3 \times 10^6$ (*sg/sg*) and $1-2 \times 10^7$ ($+/+$) cells per cerebellum. Cells were cultured in Basal Eagle's medium, supplemented with 10% horse serum (Gibco) at a concentration of 5×10^4 cells per $10 \mu\text{l}$ per microwell.

Table 2 Results of radioimmunoassay to determine cell-surface carbohydrate patterns in $+/+$ and *sg/sg* cerebellum

Antibody	Dilution	Radioactivity (c.p.m.)	
		$+/+$	<i>sg/sg</i>
Rabbit serum	1:50	20 ± 5	15 ± 7
Anti-B	1:200	50 ± 30	$16,000 \pm 1,200$
Anti-B + $10 \mu\text{g}$ meningococcus type-B polysaccharide	1:100	65 ± 15	220 ± 65
Anti- α -1,6-mannan	1:300	$12,000 \pm 300$	$2,550 \pm 275$
Anti- α -1,6-mannan + $40 \mu\text{g}$ Δ 1,6-mannan	1:100	70 ± 25	110 ± 35

P7 $+/+$ and *sg/sg* cerebellar cells were cultured as described in the legend to Table 1. After 16 h *in vitro*, cultures were washed once in ice-cold phosphate-buffered saline (PBS). $5 \mu\text{g}$ of ^{125}I -labelled purified goat anti-rabbit IgG antibodies ($\sim 8.5 \times 10^6$ c.p.m. per 14.5 mg goat IgG per ml) were added and cultures incubated at 4°C . After 2 h cultures were washed twice in 4°C PBS and once in 10% trichloroacetic acid. The tissue was then removed from the microwell and assayed for counts. Hapten inhibition was carried out as described in the legend to Fig. 1.

The pattern of cell reorganisation in the microwell cultures into cell reaggregates and connecting fibre bundles was identical in *sg/sg* and $+/+$ cultures²¹. This pattern of *in vitro* cell reorganisation was not unexpected, as *in vivo* histogenesis of *sg* cerebellum is not markedly perturbed as early as P7. Three assay systems were applied to characterise alterations of cell-surface determinants on *sg/sg* cerebellar cells in microwell cultures.

First, the results in Table 1 demonstrate fibre formation in $+/+$ and *sg/sg* cerebellar microwell culture in the presence of anti- α -1,6-mannan and anti-B antibodies. (Previous studies have shown that these antibodies do not kill living cells^{8,9}.) Fibre formation in P7 $+/+$ cell cultures was inhibited 95% with a 1:300 dilution of α -1,6-mannan antibodies, whereas the same antibody concentration inhibited only $\sim 33\%$ of fibres in *sg/sg* cultures. On the other hand, anti-B had little effect on P7 $+/+$ cultures ($\sim 15\%$), but inhibited fibre formation in *sg/sg* cultures up to 93%. The antibody reaction was polysaccharide specific, as $10 \mu\text{g ml}^{-1}$ purified type-B polysaccharide inhibited the reaction (Fig. 1), whereas polysaccharides of other specificities (dextran, pneumococcus type C carbohydrate, α -1,6-mannan) had no effect (not shown). Anti- α -1,6-mannan antibody reactivity, on the other hand, was abolished after preincubation of antibodies with yeast *Saccharomyces cerevisiae* mutant X2180-1A-5.

As discussed above, anti-B antibodies block fibre outgrowth in prenatal and neonatal but not P7 cell populations cultured in microwells^{8,9}. The inhibition by anti-B antibodies of fibre outgrowth in *sg/sg* cell cultures suggests, therefore, that 7-d-old *sg/sg* cerebellar cells express immature cell-surface carbohydrates. Although cultures are unsuccessful with cerebellum from animals older than P7, preliminary immunohistological data show corresponding cell-surface alterations in cryostat sections of *sg/sg* cerebellum as late as P18 (ref. 22).

In addition to the expression of embryonic cell-surface determinants, alteration of surface characteristics are indicated. As shown earlier⁸, anti- α -1,6-mannan antibodies did not discriminate between cells of different age in microcultures of $+/+$ but inhibited only $\sim 30\%$ of fibre formation in *sg/sg* cultures. The cell-surface determinants expressed on *sg/sg* cerebellar cells *in vitro* and *in vivo* resemble, therefore, not only immature surface antigens (as recognised by anti-B antibodies) but also constituents which seem to be structurally different from those expressed on $+/+$, as indicated by the loss of anti- α -1,6-mannan-specific antibody-binding sites.

Alterations of cell-surface carbohydrate pattern were detected also by a radioimmune assay using purified ^{125}I -labelled goat anti-rabbit IgG antibodies to detect the anti-carbohydrate antibodies bound to the surface of either $+/+$ or *sg/sg* cerebellar cells *in vitro*. The results shown in Table 2 confirmed those obtained from fibre outgrowth inhibition studies, in that alteration of cell-surface determinants on *sg/sg* cerebellar cells are in part due to alterations in cell surface carbohydrate pattern accessible to antibody recognition.

Further evidence for the expression of embryonic cell-surface characteristics on postnatal *sg/sg* cells has been obtained by studies of the effect of epidermal growth factor (EGF) on P7 *sg/sg* and $+/+$ cells cultured in microwells. As discussed elsewhere²³, EGF stimulates E16-P1 astro- and oligodendroglial cells to migrate out of reaggregates and to proliferate, but is without effect on cells older than P1. P7 *sg/sg* cells, however, respond to EGF at doses comparable to those required for E16-P1 $+/+$ cell response ($1.5 \mu\text{g ml}^{-1}$) by an increased migration and proliferation of glial cells.

Further studies will be required to elucidate the significance of the expression of immature and/or altered cell-surface molecules to the *sg/sg* mutation, and, in particular, to determine the role of cell-surface carbohydrate in the abnormal cerebellar development of *sg/sg*.

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Destruction of inferior olive induces rapid depression in synaptic action of cerebellar Purkinje cells

THE climbing fibre afferents (CFAs) are a structure unique to the cerebellar cortex; they originate, presumably solely, from the inferior olive (IO) and make an extensive, excitatory synaptic contact with dendrites of cerebellar Purkinje cells (P cells)¹. The importance of the CFAs in cerebellar functions has been emphasised in connection with the learning process which may occur in the cerebellar cortex^{2,3}, and also in connection with the development and maintenance of normal dendritic structures of P cells⁴⁻⁷. An interesting recent finding is that after destruction of the IO electrical stimulation of the cerebellar cortex fails to elicit the normal inhibitory synaptic action of P cells on their target neurones in vestibular nuclei^{8,9}. This not only urges reinterpretation of the symptoms arising from destruction of the IO^{10,11}, but also raises a basic general problem of remote interaction in neuronal networks. Here, we report that the depression of the P-cell action develops unexpectedly fast following destruction of the IO, and also present evidence indicating that this remote effect is mediated largely by a non-impulse transmission process, presumably an axonal flow, in CFAs and P-cell axons.

The vestibulospinal reflex system was chosen as the experimental material. The lateral vestibulospinal tract cells (LVST cells), located in the nucleus of Deiters, receive monosynaptic excitation from primary vestibular afferents¹² and monosynaptic inhibition from P cells in the lateral zone of the vermis of the cerebellar anterior lobe¹ (Fig. 1a). These P cells in turn receive CFAs from the caudal portion of the dorsal accessory olive (CDAO)¹³. Sixteen adult albino rabbits were anaesthetised by α -chloralose plus urethane (60 and 400 mg per kg, respectively), immobilised by gallamine triethiodide, and artificially respired. The dorsal surface of the lower medulla was exposed by opening the atlanto-occipital membrane. A part of the IO was destroyed electrolytically by passage of 0.5 or 1.0 mA d.c. currents for 1 min through a metal electrode (Fig. 1a). Location of the metal electrode in the IO was monitored by observing

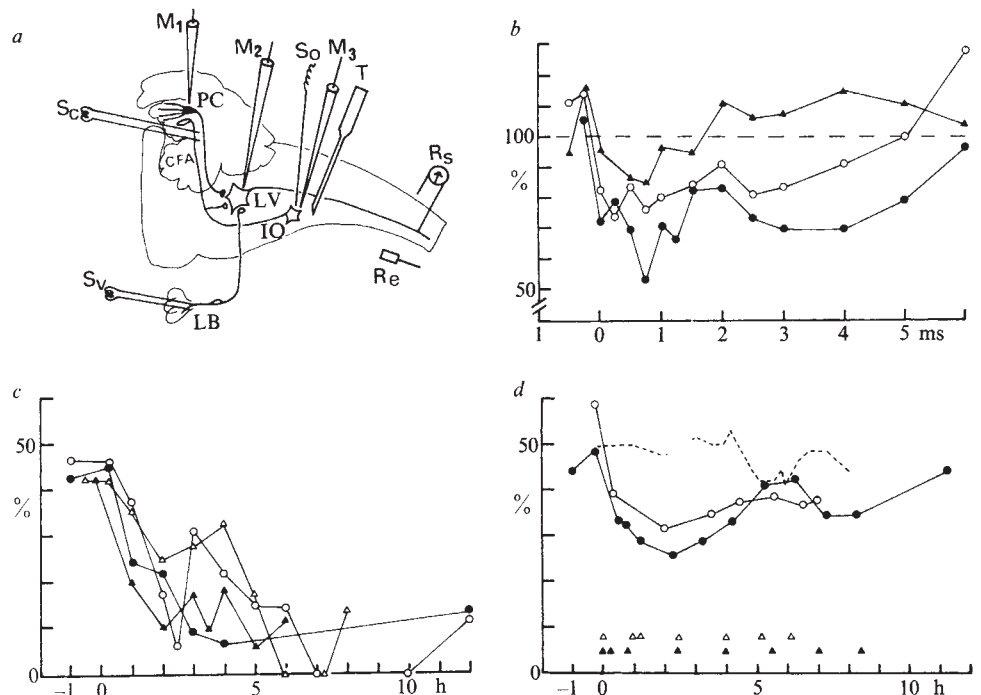
effects of stimulation through it in evoking field potentials in the nucleus of Deiters or in the vermal cortex of the cerebellum, and was later confirmed histologically.

Synchronised reflex discharges of LVST cells evoked by stimulation of the right labyrinth were recorded from the ventrolateral funiculus on the right side at the first cervical segment of the spinal cord¹⁴. These discharges were effectively inhibited by conditioning stimulation with the cerebellar electrodes placed at a deep lamella of lobule IV or V (Fig. 1a), as plotted in Fig. 1b as a function of the conditioning-testing time intervals. As demonstrated previously¹⁴, this inhibition represents the synaptic action of P cells on LVST cells. After destruction of the CDAO, the intensity of the P-cell inhibition of LVST cells gradually became much smaller (Fig. 1b). The time course of development of the depression is plotted in Fig. 1c for four experiments. In two control experiments, where lesions were placed in the rostral portion of the IO, leaving the CDAO intact, there was no depression in P-cell action such as seen in Fig. 1c (not shown).

In two experiments, IO destruction was replaced by local application of tetrodotoxin (TTX), which blocks impulse conduction but not axonal flows¹⁵. While 1 μ l of saline solution containing 1.4 μ M TTX was injected into the vicinity of the IO every hour or so, complete blockade of impulse activities in the IO was ensured by observing antidromic invasion of IO cells and/or trans-synaptic activation of P cells by stimulation of the IO. In both experiments, as demonstrated in Fig. 1d, TTX induced a rapid decline in the cerebellar inhibition but did not, however, reduce it to the extent seen after electrolytic IO destruction; instead, there was a partial recovery at 5-7 h after onset of TTX injection. This initial decline of the cerebellar inhibition was not reproduced by control injections of TTX-free saline solution, as shown by the broken line in Fig. 1d, and therefore apparently represents an effect specific to TTX.

Destruction of the IO also affected activation of P cells by CFA impulses. In Fig. 2a, the prominent negativity recorded from the molecular layer of the vermal cortex of lobule V represents trans-synaptic activation of P cells by CFAs stimulated in their intracerebellar course¹. When the CDAO was destroyed together with the caudal portion of the medial accessory olive which projects to the medial part of the vermis¹³, the activation of P cells gradually became much less effective in a

Fig. 1 Effects of destruction of, and local application of TTX to, the IO on P-cell inhibition of LVST cells. *a* illustrates schematically the experimental arrangements on a parasagittal section of the cerebellum and brainstem. PC, P cell; LV, LVST cell; LB, labyrinth; S_v, S_c, S₀ and R_s, platinum-iridium needle electrodes, 0.2 mm in diameter, lacquered except for the very tips. One needle of R_s was inserted into the spinal cord, the other was placed on neck muscles. S₀ was used for electrolytic destruction of IO. M₁, M₂ and M₃, glass microelectrodes filled with 2 M NaCl solution and having electric resistance of 1.5-2.5 M Ω . M₁ and M₂ were for recording from the cerebellar cortex and the nucleus of Deiters, respectively. M₃ was for examining antidromic activity of IO neurones under the influence of TTX; T, microsyringe containing 1.4 μ M TTX-saline solution; R_e, indifferent electrode. *b* Plots amplitudes of LVST-cell discharges evoked by testing stimulation with S_v and recorded with R_s (ordinate), relative to their control size, as a function of the time interval between the conditioning stimulation with S_c and the testing one (abscissa). Three sets of plotted points were obtained before (●), and 4 (○) and 12 (▲) h after IO destruction in the same experiment. *c* Plots the reduction in LVST-cell discharges at the peak of the inhibition curves as in *b*, relative to their control size, as a function of time. IO destruction ended at 0 h. Results from four animals are superimposed. *d* Is similar to *c*, except for TTX application. For the two cases plotted by ○ and ●, times of TTX injection are indicated by Δ and $\blacktriangle$, respectively. The broken line is for the control case where normal saline solution was injected every hour starting at 0 h.



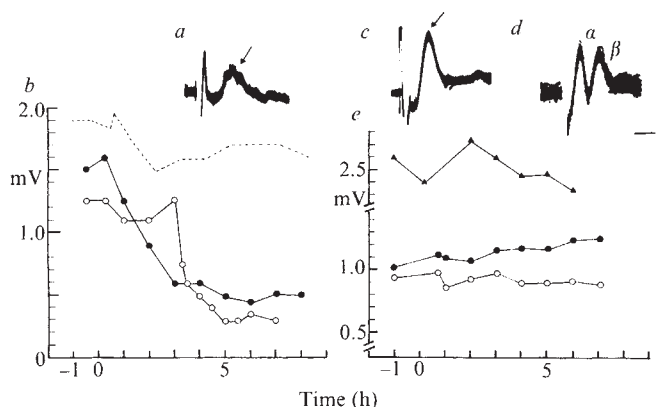


Fig. 2 Effect of IO destruction on CFA activation of P cells and electrical excitability of P-cell axons. *a*, Field potentials evoked by applying pulses of 0.1-ms duration and 0.5-mA intensity through S_c and recorded with M_1 (see Fig. 1*a*). Arrow marks the negativity representing activation of P cells by CFAs. *b*, Peak amplitudes for two animals plotted as a function of time before and after destruction of IO which ended at 0 h. The broken line indicates CFA activation of P cells in the presence of TTX, as shown in Fig. 1*d* (●, ▲). *c*, Field potentials evoked in the Purkinje cell layer by stimulation similar to *a* and recorded with M_1 optimally adjusted for recording antidromic activity of P cells (marked by an arrow). *d*, Field potentials evoked in the nucleus of Deiters by stimulation similar to *a* and *c*. α and β denote two spiky peaks. *e*, Plots amplitudes of antidromic activity of P cells (—▲—) and of α (●) and β (○) spikes, obtained from two animals as a function of time as in *b*. Records *a*, *c* and *d* were obtained by superimposing five successive sweeps repeated at a rate of 5 s⁻¹. For the measurement of these potentials for plotting in *b* and *e*, an average of over 20 successive sweeps was routinely done by use of a data processing computer (ATAC 501-10). Time scale indicated in *d* is 2 ms for *a* and 0.4 ms for *c* and *d*. Voltage scale is 1 mV for *a* and 0.4 mV for *c* and *d*.

time course comparable to that for depression of the cerebellar inhibition of LVST-cells, plotted in Fig. 2*b* for two cases. Local application of TTX induced only a small, somewhat transient depression in the activation of P cells, as indicated by a broken line in Fig. 2*b*.

By contrast, the excitability of P-cell axons was not affected by IO destruction. As shown in Fig. 2*c*, cerebellar stimulation by electrode S_c evoked a prominent negativity in the P-cell layer of lobule V which represented antidromic invasion of P cells. Field potentials similarly evoked but recorded from the nucleus of Deiters contained two spiky components of fast negativity (Fig. 2*d*), the earlier α spike representing volleys evoked in cerebellar afferent fibres and the later β spike those in P-cell axons¹. Except for relatively small changes presumably arising from inadvertent movement of the recording electrode relative to brain tissues, none of these potentials exhibited systematic change following destruction of the IO (Fig. 2*e*).

The present investigation demonstrates that destruction of IO neurones causes a rapid failure in the excitatory transmission from CFAs to P cells and in the inhibitory transmission from the latter to LVST cells. These effects are specific to portions of the IO related to the vermal P cells and LVST cells, thus excluding the possible contribution of a nonspecific effect of damage to the medulla. As blockade of impulse activities of the IO with TTX produced only a relatively small, transient effect (Fig. 1*d*), the major effect of the IO destruction must be mediated by a non-impulse process, presumably an axonal flow in the CFAs. As impulse conduction in P-cell axons was not affected by IO destruction, it seems also that the postulated disturbance of axonal flow in CFAs affects the axonal flow in P-cell axons across CFA-P-cell synapses, eventually resulting in reduction of either release of the neurotransmitter, presumably GABA¹⁶, from P-cell axon terminals, or postsynaptic reactivity to the neurotransmitter. It may be that P-cell synapses on LVST cells are affected through collateral of CFAs directly innervating LVST cells in the manner of the heterosynaptic interaction (see Fig. 1*a*).

Fast components of axonal flow have a velocity as high as 400 mm d⁻¹ (ref. 17), and may travel from the IO to LVST cells through P cells in about an hour. This is fast enough to account

for the rapid transfer of the effect of IO destruction to LVST cells, except for the short-term effect revealed within the initial hour (Fig. 1*c*). The short-term effect could be due to removal of impulses from CFAs and/or tissues surrounding the IO, as obtained by TTX blockade; P-cell inhibition of LVST cells might be influenced rapidly through a process such as impulse-axonal-flow interaction¹⁸ in CFAs and/or P-cell axons, or through alteration of background activities of LVST cells, such as removal of the inhibition arising from the medullary reticular formation¹⁹.

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Transducer noise in a photoreceptor

IN our attempts to unravel the workings of visual systems, we seek neural responses and interactions which can account for visual behaviour. A popular measure of visual performance is the threshold intensity at which a stimulus is detected with a given probability. To account for these detection tasks, one must not only measure neural signals at threshold^{1,2}, but also find the limiting sources of variance (that is, the noise) both within³ and outside^{4,5} the nervous system⁶. The manner in which the random nature of photon absorptions can limit the performance of visual systems is well understood^{4,5,7,8}. When bleaching effects are negligible, photon absorptions follow the Poisson distribution. Consequently photon counts derived from the same mean signal have a variance equal to their mean. These fluctuations in counts are an inherent property of photon signals and are referred to as photon shot noise. Processes within the visual system generate intrinsic noise⁷, but to assess its effect on thresholds one must first account for photon shot noise. This is generally difficult because photon catch cannot be measured directly, and must be estimated from optical parameters which are subject to significant error⁷. We have estimated the levels of photon shot noise and intrinsic transducer noise⁹ in locust photoreceptors at low light intensities. We have chosen to search for sources of intrinsic noise in the visual system of the locust because it has recently been shown that, at low intensities, each effective photon produces a single, large quantum bump¹⁰ (Fig. 1). Thus, we measure precisely the number of photons contributing to photoreceptor signals and derive, from the total response variance, that additional variance due to intrinsic noise. We report here that the transducer noise mimics the properties of photon

Table 1 Analysis of photoreceptor response variance

Cell no.	Total response variance ($\bar{N}_c$) ²	Mean effective photons per flash ($\bar{N}_c$) = predicted variance due to photon shot noise ($\bar{N}_c$) ²	Excess response variance due to transducer noise ($\bar{N}_c$) ²	% Total	Mean response amplitude (mV)
1	10.0	5.8	4.2	42.0	6.4
	14.8	8.9	5.9	40.0	7.4
	24.1	14.1	10.0	41.4	9.2
	32.3	21.4	10.9	33.7	10.0
	69.0	31.6	37.4	54.2	11.7
2	448.5	174.9	273.6	61.0	16.1
	5.3	3.3	2.0	38.0	10.5
3	7.2	3.3	3.9	54.0	12.0
	27.9	9.2	18.7	67.0	17.0
	41.0	16.4	24.6	60.0	18.9
4	7.1	4.7	2.4	33.5	5.0
	27.6	13.5	14.1	51.0	9.0
5	25.9	11.4	14.5	56.0	6.4
6	21.4	10.5	10.9	51.0	10.0
7	65.3	27.8	37.5	57.4	11.9
8	25.3	14.8	10.5	41.4	7.0
	48.5	24.7	23.8	49.1	7.9
	88.5	37.7	50.8	57.4	10.9
Mean = 49.3 ± 9.9%					

shot noise, is of similar magnitude, and, in most conditions, must have an equally significant role in determining thresholds.

To examine photoreceptor noise we use techniques perfected in previous experiments⁹⁻¹² and estimate receptor performance from intracellular responses to brief monochromatic light flashes (4 ms at 443 nm). Each stimulus falls within the linear integration time of the photoreceptor (which is >10 ms in a

dark-adapted locust) so that response amplitude depends on the total number of effective photons delivered. Thus, the photoreceptor is used as a photon counter and problems associated with the statistical analysis of rate-dependent processes are avoided.

For each photoreceptor analysed we first count the number of bumps produced during prolonged periods of dim illumination.

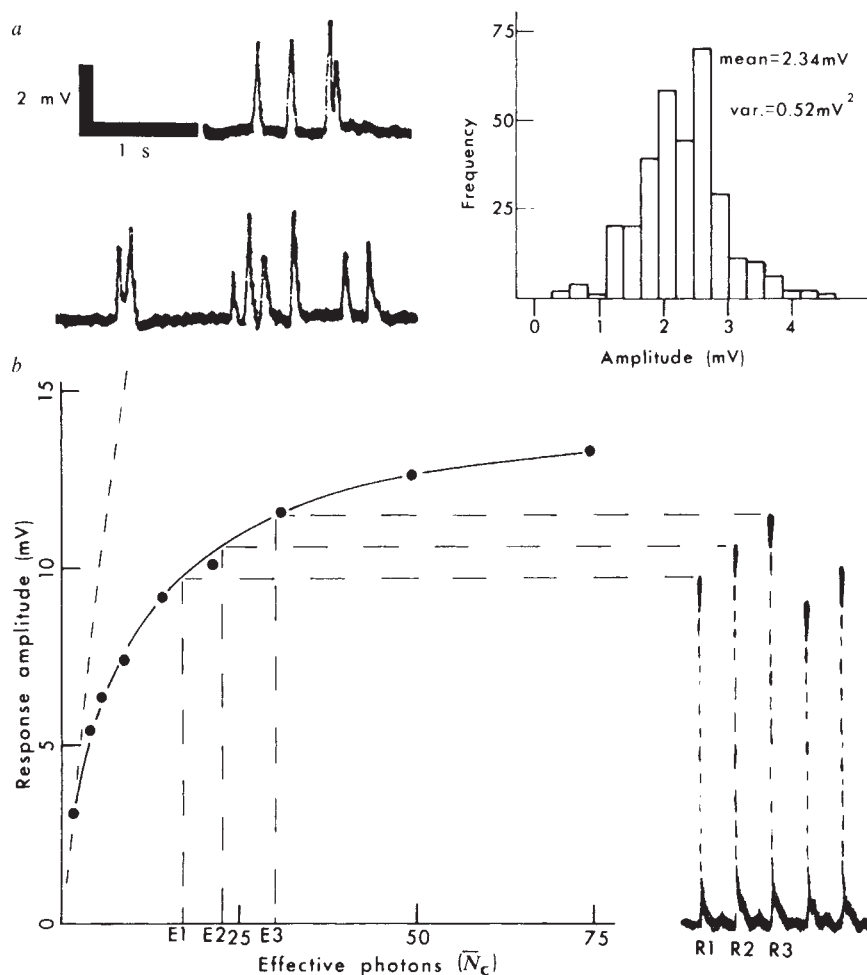


Fig. 1 Measurement of transducer noise in locust photoreceptors. *a* Shows quantum bumps recorded during dim illumination and their amplitude distribution. Their frequency is used to calibrate the effective quantal content of the flashes used to estimate response variance. *b* Demonstrates how an equivalent number of captured photons (E1, E2, E3, and so on) is attributed to responses (R1, R2, R3, and so on) induced by brief, higher-intensity flashes by referring all amplitudes to the intensity/response function. The intensity/response function predicted by the simple self-shunting model (---) is much steeper than the empirical (●—●).

This enables us to calculate the mean number of effective photons, $\bar{N}_e$, contained in higher-intensity flashes. Next, we derive the intensity/response function relating mean response amplitude, $\bar{V}$, to $\bar{N}_e$ (Fig. 1). Finally, we measure the amplitudes of response to 100–200 repeated flashes of known mean effective quantal content. Photon and intrinsic noise sources cause these responses to fluctuate in amplitude (Fig. 1) and response variance is measured in units of equivalent effective photons (EEPs) squared by converting every voltage amplitude to an equivalent mean photon count^{9,12} using the intensity/response function (Fig. 1). The response variance due to photon shot noise equals $\bar{N}_e$ (from Poisson statistics). The additional response variance due to intrinsic noise is derived by subtracting the photon shot noise from the total response variance. Thus, the degrading effects of intrinsic noise are measured in terms of incident intensity.

Our results (Table 1) show that over a range of 1.7 log units intensity, the random absorption of photons only accounts for 33–67% of total response variance. The remaining fluctuations must be generated within the photoreceptor itself. At low intensities this intrinsic noise is multiplicative, producing variance proportional to mean photon catch, $\bar{N}_e$, in the range $\bar{N}_e = 6$ –30. At higher intensities our preliminary observations suggest that intrinsic noise increases in magnitude relative to photon shot noise (Table 1).

We are confident that this intrinsic noise is not an artefact generated by errors in experiment or calibration. Full details of the calibration of the Uniblitz high speed shutter, the constant current tungsten light source, and the interference and attenuating filters are given elsewhere¹⁰. Calibrations gave consistent results which accurately predicted both relative quantum bump frequencies at different steady intensities and the fraction of successful responses produced by different threshold flash intensities. The accuracy of our method depends on the determination of relative, rather than absolute, stimulus energies, because each cell's quantum catch is measured from bump counts. Rigorous controls ensured that we rejected data from cells whose sensitivity changed during an experiment. A determination of quantum capture efficiency and the intensity/response function preceded and followed every estimation of response noise, which was measured several times at each intensity. The inter-stimulus interval was adjusted to prevent light adaptation. During a single experiment, the resting potential drifted by <0.5 mV (~1%).

We can dismiss several sources of intrinsic noise in the dark-adapted locust photoreceptor. In total darkness, 'spontaneous' photon signals occur at a rate of less than 1 every 360 s (ref. 10) (this is 1 every 160 s for dogfish rods¹³) and will contribute negligible variance to responses to brief flashes. Membrane voltage noise in the dark (400 μ V peak-to-peak) is also negligible relative to bump amplitude and this contrasts strongly with the barrage of dark noise in rods and cones¹⁴. Consequently, in locust photoreceptors, the intrinsic noise described above must be associated with the light response, and so is termed transducer noise⁹. One of us (P.G.L.) has recently eliminated three potential sources of this transducer noise: there is no variance associated with the number of bumps produced by an effective photon¹⁰; bump size seems to be independent of photon wavelength¹¹, even in cells containing two or more photopigments, implying that each photopigment produces equal-sized bumps; there is no variance associated with site of photon capture (geometric noise).

Two known manifestations of transducer noise are the fluctuations in amplitude (Fig. 1) and latency of single photon responses¹⁰. With respect to amplitude fluctuations, the simplest assumption is that apart from overall response compression, bumps add independently to each other. On this basis one would expect the slope, S , of variance against mean (in terms of EEPs) to be: $S = 1 + (\text{variance}(\text{bump amplitude})/(\text{mean}(\text{bump amplitude}))^2)$ (see ref. 15), which is always less than observed; for example, the predicted slope for cell 1, Table 1 is 1.1 rather than the observed value of 1.5–2.0. The disparity presumably

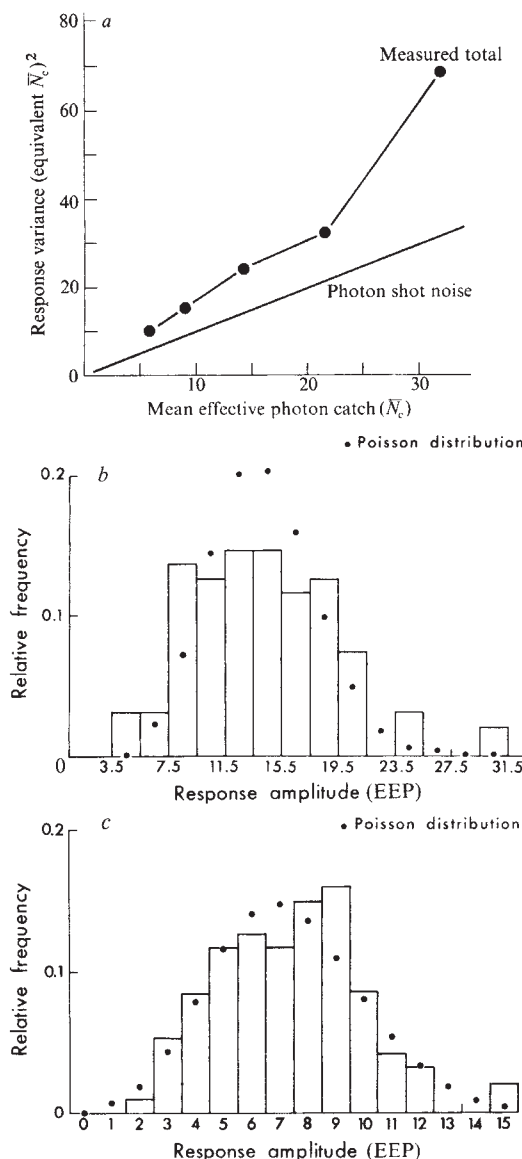


Fig. 2 *a*, Total response variance measured in squared equivalent effective photons (EEPs) plotted against mean effective photon catch per flash. For small responses the total variance is proportional to mean intensity and in this sense mimics photon shot noise. *b*, The frequency distribution of response amplitudes to one series of constant-intensity flashes. Response amplitudes are expressed in EEPs using the methods described in the text. The distribution of amplitudes is broader than that predicted by Poisson statistics from the mean effective photon catch estimated from bump counts (●). This extra scatter is due to transducer noise. *c*, An amplitude frequency distribution plotted from the same data as used in *b*, but here the shot noise analysis described in previous studies^{9,12} has been used to assign to each response an effective quantal content. This method assumes that all variance is generated by photon shot noise and calculates the mean quantal content of stimuli from the signal-to-noise ratio of response amplitudes⁹ (expressed in equivalent intensity). Again, response noise mimics photon shot noise because the distribution of apparent photon catches is Poissonian. However, intrinsic noise has decreased the signal-to-noise ratio and lowered the apparent photon catch. All data are taken from cell 1, Table 1.

indicates that such a simple assumption is not justified. This is suggested by the observation that locust photoreceptors do not possess 'simple' self-shunting membranes (for review see ref. 16) for which the above equation for slope, S , was derived¹⁵. In this case, total conductance change is proportional to photon catch and a hyperbolic intensity/response function is predicted which greatly overshoots the empirical one (Fig. 1). Thus, a better understanding of the mechanisms which produce this

severe response compression seems necessary to assess the effects of bump amplitude variability. Initial modelling of the effects of variable bump latency using Monte Carlo methods suggests that it causes very little response compression and contributes about 10% to observed transducer noise. Clearly, we require a better knowledge of transduction if we are to determine the extent to which the unequal responses to single photon absorptions seen from bumps, contributes to transducer noise.

Can transducer noise influence neural and behavioural thresholds? At intensities so low that bumps do not fuse, single photon hits could be registered in a manner which avoids transducer noise. For example, given the high voltage gain found at the first synapse in the compound eye (reviewed in ref. 17), the second-order cells might produce brief saturating responses for each photoreceptor bump received. However, at higher intensities, bumps fuse and a receptor can no longer act as a perfect photon counter in this manner. Transducer noise blurs the discrete jumps in amplitude resulting from additional photon absorptions, and contributes noise indistinguishable in the intensity domain from photon shot noise (Fig. 2*b, c*). Arguments which suggest that the proportionality of increment thresholds to (intensity)^{1/2} indicate a photon-counting system unaffected by intrinsic noise⁸, are here demonstrated to be false, because we describe a source of intrinsic noise (transducer noise) which is multiplicative. Thus, although photoreceptor noise has a large intrinsic component, it mimics the statistical properties expected if photon shot noise alone was present⁶. Our measurements suggest that in most conditions transducer noise must be as significant as photon shot noise in determining visual thresholds. Consequently, transducer noise must be considered with other sources of intrinsic noise when accounting for visual performance, for the design of photoreceptors, and for higher-order strategies for optimum sampling of the visual signal¹⁸.

During the revision of this letter, a report was published which suggested that multiplicative noise might degrade human visual performance without distorting the underlying Poisson statistics of photon capture. This would account for the number of quanta apparently detected falling far short of estimated capture by the photopigment¹⁹. Our conclusions about the effects of transducer noise are clearly similar and establish a precedent for the existence of multiplicative noise within a visual system.

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Association of cellular membrane of *E. coli* minicells with the origins/terminus region of replication of plasmid ColEI DNA

COLICINOGENIC plasmid EI (ColEI) can be isolated from *Escherichia coli* as a supercoiled DNA molecule of 2.14 μm long or as a supercoiled DNA–protein complex¹. Certain protein denaturing agents will convert this complex to an open circular (relaxed) DNA form with one single-strand break in the heavy (polyUG binding) strand of the duplex ColEI DNA (refs 2, 3). The induced single-strand break in ColEI DNA is located approximately 19% of the length of ColEI DNA from the single *Eco*RI restriction endonuclease site in the plasmid⁴. Analysis of ColEI replicating intermediates has shown that replication proceeds unidirectionally from an origin located near the position of the relaxation complex nick, approximately 19% from the single *Eco*RI restriction site^{5–7}. The site of the relaxation-complex nick has been located approximately 300 nucleotides away from the origin of ColEI DNA replication⁹. The nucleotide sequence of the origin region of ColEI DNA^{8,9} and of a small ColEI-type plasmid¹⁰ have been determined. A model for the possible role of the relaxation complex in ColEI DNA replication and/or conjugal transfer has been presented¹¹. A major feature of this model is that the relaxation complex proteins may help promote association of plasmid DNA with a replicator and/or conjugal transfer site on the bacterial membrane. We describe here an electron microscopic analysis of *Eco*RI-cleaved ColEI and mini-ColEI DNA that were purified as stable DNA–membrane complexes from *E. coli* minicells. Evidence is presented that membrane-like structures attach to ColEI DNA and mini-ColEI DNA in a region that includes both the site of the origin/terminus of DNA replication and the site of single-strand cleavage by the relaxation complex.

Minicells that contain plasmid ColEI DNA or a hybrid mini-ColEI plasmid containing a kanamycin (Km) resistance fragment (pML21)¹³ were purified from *E. coli* P678–54, Thy[–]. The plasmid DNA in the purified minicells was labelled with ³H-thymidine, the minicells were lysed, and the lysate centrifuged on caesium chloride–sucrose gradients¹². Figure 1*a* shows that in the conditions of centrifugation the ³H-ColEI DNA from minicells separates into two major peaks, one which rapidly sediments to an isopycnic density of about 1.35 g ml^{–1} (pool A), the other remaining near the top of the gradient. A visible band of opalescent material, presumably cellular membrane fragments, is present in the position of pool A. To show that pool A contains membrane components, cultures of *E. coli* P678–54 harbouring ColEI were labelled with ³H-leucine or [^{2-³H}]glycerol, which are incorporated into protein and membrane phospholipid, respectively¹⁴. Minicells were purified from these cultures and treated exactly as before, except the cells were not labelled with ³H-thymidine. It was found (data not shown) that 17% of the total ³H-leucine in the lysed minicells is located in the 1.35 g ml^{–1} density region in the caesium chloride–sucrose gradient. In addition, 100% of the phospholipid label reached equilibrium at a density of 1.35 g ml^{–1}. Therefore, the observed isopycnic density of the ³H-ColEI DNA is most probably accounted for by the association of the plasmid DNA with membranous components.

Purified supercoiled, open circular, or linear ¹⁴C-ColEI DNA was added to the lysate at various stages of the lysis procedure. Figure 1*a* shows that supercoiled ¹⁴C-ColEI DNA does not appear in the pool A region of the gradient, suggesting that little or no ³H-ColEI DNA from the minicell lysate aggregated nonspecifically to components in this peak. Similar results occurred with purified circular and linear ¹⁴C-ColEI DNA. As shown in Fig. 1*b*, when pool A was dialysed and centrifuged a second time on a caesium chloride–sucrose gradients, approximately 50% of ³H-ColEI DNA in pool A remained near the top

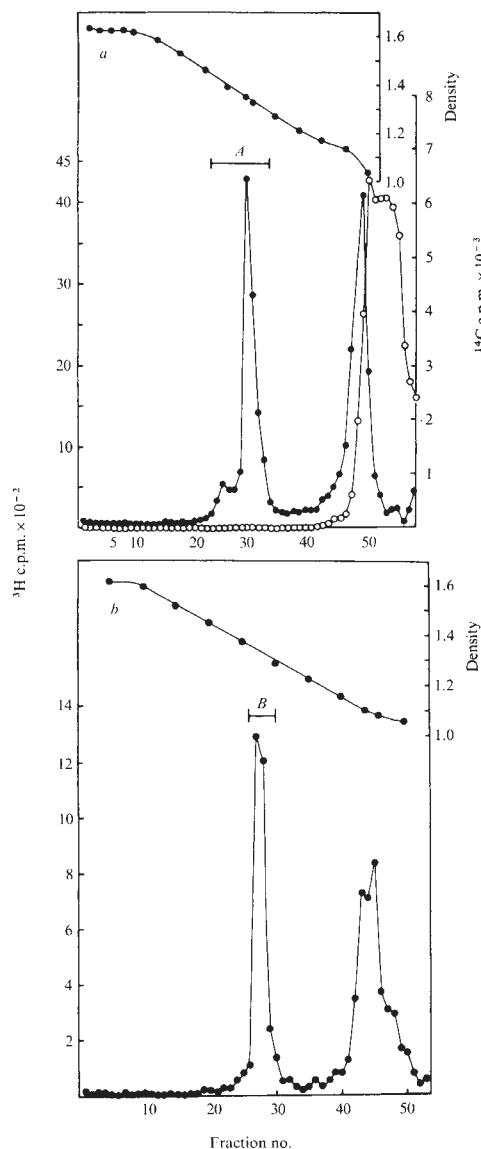


Fig. 1 Caesium chloride-sucrose gradients of lysates of minicells containing ^3H -labelled plasmid ColEI DNA. *a*, *E. coli* minicell producing strain, P678-54, Thy^- , carrying plasmid ColEI was grown to late exponential phase in 3 l of LB medium. The culture was centrifuged at 1,500 r.p.m. for 3 min in a Sorvall GSA rotor and the supernatant pelleted at 8,000 r.p.m. for 10 min, followed by resuspension in BSG (0.85% NaCl, 0.03% KH_2PO_4 , 0.06% Na_2HPO_4 , 0.1% gelatin). The resuspended minicells were layered on 33 ml 5–20% sucrose gradients and centrifuged in an HB4 rotor at 4,500 r.p.m. for 15 min. The minicell band was removed, diluted with BSG, pelleted, and again centrifuged on 5–20% sucrose gradients. This was repeated 2 or 3 times until *E. coli* whole cells could no longer be seen in the minicell preparation by light microscopy. ColEI DNA in the purified minicells was labelled with ^3H -thymidine by incubation for 30 min at 37°C in 25 ml of M9 medium containing 0.1% vitamin-free casamino acids, 0.2% glucose, $20\text{ }\mu\text{g ml}^{-1}$ thiamine, $50\text{ }\mu\text{g ml}^{-1}$ tryptophan, 0.002 M cyclic AMP and $2\text{ }\mu\text{g ml}^{-1}$ each of adenosine, deoxyadenosine, guanosine, deoxyguanosine, cytosine, deoxycytosine and uridine, and 1 mCi of ^3H -thymidine. Incorporation of ^3H -thymidine into trichloroacetic acid-insoluble material was linear up to 90 min. After labelling, the minicells were centrifuged and resuspended in 2 ml of 0.75 M sucrose. Purified ^{14}C -thymine-labelled ColEI supercoiled DNA was added immediately after sucrose as a control for nonspecific association of ColEI DNA with membrane components. The mixture was incubated for 15 min on ice with $100\text{ }\mu\text{g ml}^{-1}$ lysozyme and 0.01 M EDTA, after which Triton X-100 was added to 0.05%. The lysate was layered on a preformed linear gradient of 5 M caesium chloride plus 20% sucrose to 0.5 M caesium chloride plus 10% sucrose made in TES (0.1 M Tris, pH 8.0, EDTA, pH 8.0, 0.5 M NaCl, 0.005 M) and centrifuged in an SW 27 rotor at 22,000 r.p.m. at 4°C for 30 min¹². Fractions were collected from the bottom, portions counted, and the refractive index of each fraction determined. The density was determined from caesium chloride-sucrose standard curves of density against refractive index. ●, ^3H radioactivity; ○, ^{14}C radioactivity. *b*, Pool A from the caesium chloride-sucrose in *a* was dialysed against TES and centrifuged on a caesium chloride-sucrose gradient in the same conditions as above.

of the gradient. Dialysis and centrifugation of pool *B* (Fig. 1*b*), again in the same conditions, does not result in any loss of ColEI DNA from the 1.35 g ml^{-1} density region, indicating that the remaining 20–25% of the total radioactivity incorporated into ColEI DNA (pool *B*) is stably associated with membrane material. In different experiments the percentage of total ^3H -thymidine incorporated into ColEI DNA found in pool *A* (Fig. 1*a*) varied, but the percentage of incorporated ^3H -thymidine remaining in pool *B* (Fig. 1*b*) was usually 20–25%. The released ^3H -ColEI DNA near the top of the gradient may represent plasmid DNA either specifically but weakly associated with membrane components or nonspecifically-associated DNA that is released during handling.

As a further test of the stability of ^3H -ColEI DNA membrane association, pool *B* was subjected to various treatments. It was found that ColEI DNA can be released from membrane material, as determined by a change in density of the ^3H -DNA in caesium chloride-sucrose gradients, by treatment with pancreatic DNase or the ionic detergents Sarkosyl and SDS (0.2%). Treatments that did not release the plasmid DNA included exposure of 2M NaCl, Triton X-100 (0.2%), pancreatic RNase (1 mg ml^{-1}), phospholipase A, freezing to -20°C , and heating to 60°C for 10 min. Treatment with pronase or heating at 100°C for 10 min. gave variable levels of dissociation of the complex.

ColEI DNA-membrane complexes from pool *B* (Fig. 1*b*) and mini-ColEI-Km (pML21) DNA-membrane complexes from similar preparations were examined by electron microscopy.

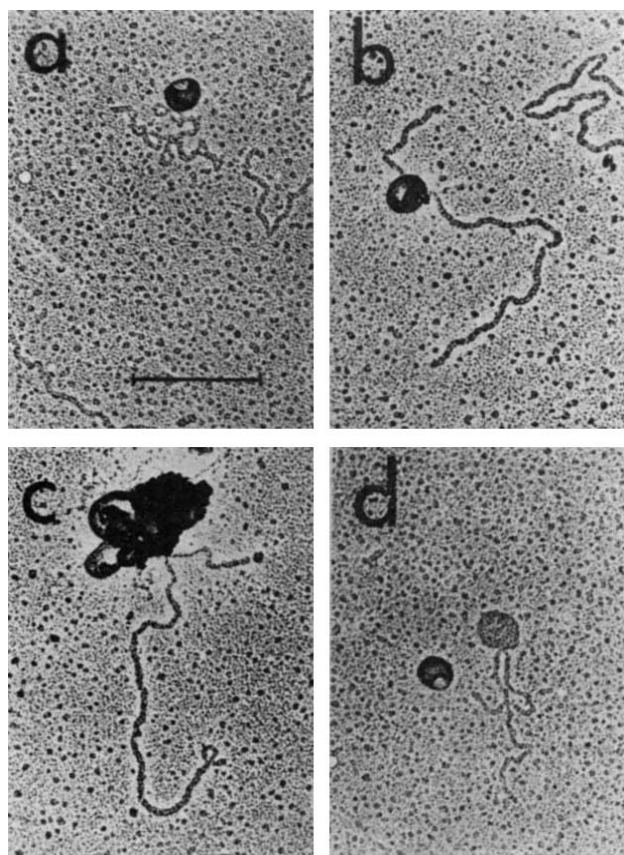


Fig. 2 Electron micrographs of *Eco*RI-cleaved ColEI DNA-membrane complexes and mini-ColEI DNA-membrane complexes. Minicells containing ColEI DNA or mini-ColEI-Km DNA were purified, labelled, lysed and centrifuged on caesium chloride-sucrose gradients as described in Fig. 1. Fractions corresponding to Pool *B* in Fig. 1 were dialysed against TES. Aliquots were adjusted to 10 mM MgCl_2 and either incubated at 30°C for 30 min as controls for nuclease contamination or incubated at 30°C for 30 min with *Eco*RI. Control and cleaved samples were spread for electron microscopy as previously described¹⁶. Purified open circular ColEI DNA was spread with the samples as a size reference. *a*, Supercoiled ColEI DNA-membrane complex; *b*, *c*, *Eco*RI-cleaved ColEI DNA-membrane complex; *d*, *Eco*RI-cleaved mini-ColEI DNA-membrane complex. The bar in frame *a* is equal to $0.5\text{ }\mu\text{m}$.

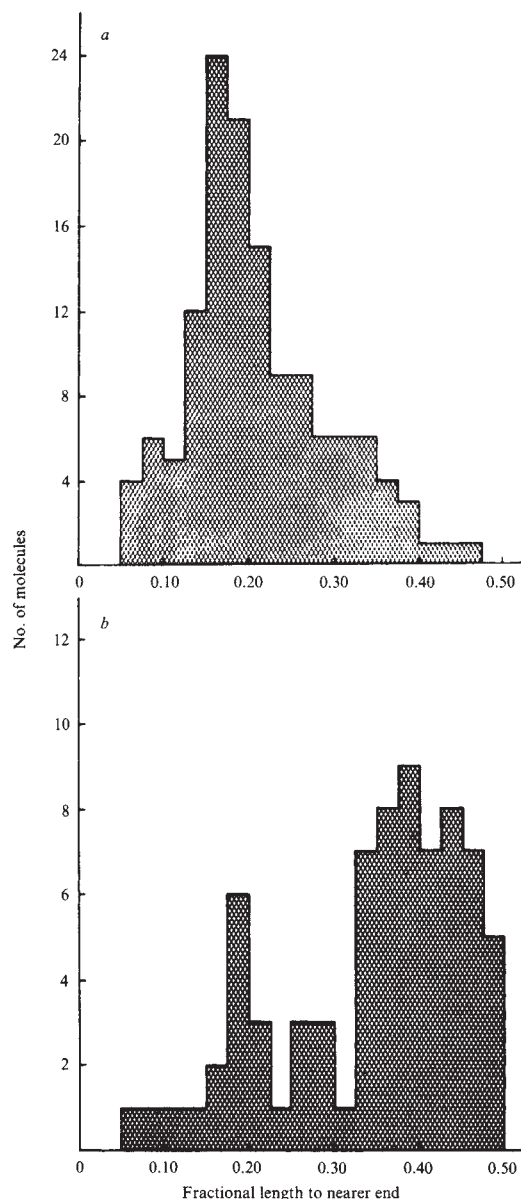


Fig. 3 Histogram of locations on *Eco*RI-cleaved ColEI DNA and mini-ColEI DNA in contact with a membrane fragment. Samples of purified ColEI DNA or mini-ColEI-Km DNA analogous to pool B of Fig. 1 were cleaved with *Eco*RI and visualised by electron microscopy. Molecules were measured in relation to purified circular ColEI DNA spread on the same grid, and only molecules that were of unit length were considered for analysis. Measurements were made on the short arm of the restricted DNA from the *Eco*RI site to the membrane fragment. Molecules with large membrane fragments covering the DNA in more than one of the arbitrary divisions of 0.025 molecular length were included in the histogram in each division in contact with membrane material. *a*, ColEI DNA; *b* mini-ColEI-Km DNA.

Figure 2 shows complexes before and after cleavage by *Eco*RI restriction endonuclease. This analysis usually showed some membrane-like fragments free from DNA in addition to supercoiled, open circular, and, in the case of restricted samples, linear plasmid DNA membrane complexes. Over 50% of the linear ColEI DNA observed was associated with a membrane fragment. A low percentage (<5%) of the membrane-bound ColEI DNA had two fragments attached. Most DNA-bound membrane fragments appeared vesicular and seemed to be of two general types (Fig. 2*a, b, d*). These fragments were about 0.1 μ m in diameter. Other larger fragments could also be seen (Fig. 2*c*). When pool B was treated with 0.2% Sarkosyl and dialysed before preparation for electron microscopy, membrane-like structures could not be seen. As mini-ColEI-Km

DNA has two *Eco*RI restriction sites, restricted preparations showed two molecules, the 4.5×10^6 -dalton Km restriction fragment and the 2.2×10^6 -mini-ColEI plasmid. Less than 3% of the Km DNA fragment possessed an attached membrane fragment. However, a large proportion of the mini-ColEI DNA was associated with a membrane fragment.

The total lengths of the linear molecules associated with membrane fragments derived from *Eco*RI-cleaved DNA of pool B were determined by electron microscopy. For ColEI DNA, only molecules that were the same length as open circular ColEI DNA spread on the same grid square were further analysed. For mini-ColEI only linear DNA molecules that were 1.2 μ m in length when compared to the control ColEI molecules spread on the same grid square were further analysed. The location on the DNA molecule of the membrane fragments relative to the *Eco*RI restriction sites was measured. A total of 75 ColEI molecules from three different preparations and 50 mini-ColEI molecules from two different preparations were measured. Figure 3*a* and 3*b* represent, respectively, the number of ColEI and mini-ColEI molecules that had a membrane fragment in contact with the DNA within arbitrarily chosen divisions of 0.025 fractional length of the DNA molecule. If a membrane fragment was in contact with a DNA molecule in more than one of the divisions of 0.025 fractional length it was counted and plotted in each division. Some of the scatter and broadness of the histogram peaks may be due to nonspecific associations or to large membrane fragments that cover disproportionately large regions of the DNA molecules. As salt concentrations of ≥ 2 M will dissociate most DNA binding proteins¹⁵, the caesium chloride-sucrose gradient conditions probably dissociate most weak associations.

Figure 3*a* indicates that about 60% of the ColEI DNA-bound membrane fragments are in contact with the DNA between 0.15–0.22 fractional length of the *Eco*RI restricted plasmid. This corresponds to the region of origin/terminus of replication and to the relaxation complex site of ColEI DNA. Figure 3*b* shows that about 55% of mini-ColEI DNA-bound membrane fragments are found at 0.33–0.45 fractional length of the *Eco*RI cleaved molecule. This is in the region of the origin of DNA replication of the mini-ColEI molecule (0.36–0.40 fractional length of *Eco*RI-cleaved mini-ColEI (R.B.S. and R. Eichenlaub, unpublished). Although this analysis does not distinguish between the two ends of the DNA molecules, the close correspondence to the origin of replication suggests a direct involvement of membrane components in plasmid ColEI DNA replication. The failure to isolate mini-ColEI DNA in the form of relaxation complex¹³ may indicate in the case of this plasmid that the association with the membrane material is more a property of the origin/terminus of replication or the relaxation complex site of nicking than of the active relaxation complex proteins.

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A peptide resembling COOH-terminal tetrapeptide amide of gastrin from a new gastrointestinal endocrine cell type

THE gastrointestinal hormones gastrin and cholecystokinin (CCK) share identical COOH-terminal sequences. In both hormones the COOH-terminal tetrapeptide amide constitutes the biologically active region, and the rest of the two molecules determines their potency and selectivity for different target cells. Their similarity in structure and activity may reflect a common phylogenetical origin¹. The COOH-terminal tetrapeptide amide is highly immunogenic in each hormone and many gastrin antisera are specific for this sequence and also bind CCK. In order to study the hormones by immunocytochemistry and radioimmunoanalysis, antisera specific for several distinct sequences must be used. Previously, we have used such antisera to characterise antral gastrin cells and intestinal cholecystokinin cells by immunocytochemistry². We now report the identification of three distinct types of gastrointestinal cells which show COOH-terminal gastrin (or CCK) immunoreactivity. One type corresponds to gastrin-producing G cells, another to CCK-producing I cells and a third type to TG cells which react only with antisera against the COOH-terminal tetrapeptide.

Specimens from the gastrointestinal tract of monkeys ($n = 5$) and pigs ($n = 10$) were studied by immunocytochemistry and correlative electron microscopy. The antisera used included As. 1295, 2717 and 4562, raised in rabbits against synthetic human gastrin-17, As. 4710 raised in a rabbit against the synthetic mid-portion (6-13) of human gastrin-17, As. 4478 raised in a guinea pig and As. 4698 raised in a rabbit against porcine CCK-33. As previously documented^{1,2} As. 1295 recognises the NH₂ terminus of human gastrin-17, As. 4710 the mid-portion of human gastrin-17, As. 4562 and 2717 bind the COOH-terminal tetrapeptide portion of both gastrin and CCK, As. 4698 binds the region (25-30) of triacontatriapeptide CCK (CCK-33) and so also recognises octapeptide CCK (CCK-8) and caerulein, and As. 4478 binds the region (19-25) of CCK-33 and does not recognise CCK-8. The region-specificity of the antibodies is

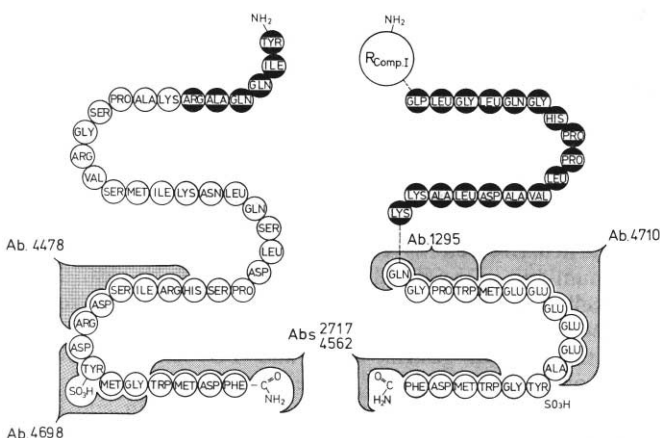


Fig. 1 The amino acid sequences of porcine CCK-33 and gastrin-34 and the region-specificity of the antisera used in this study.

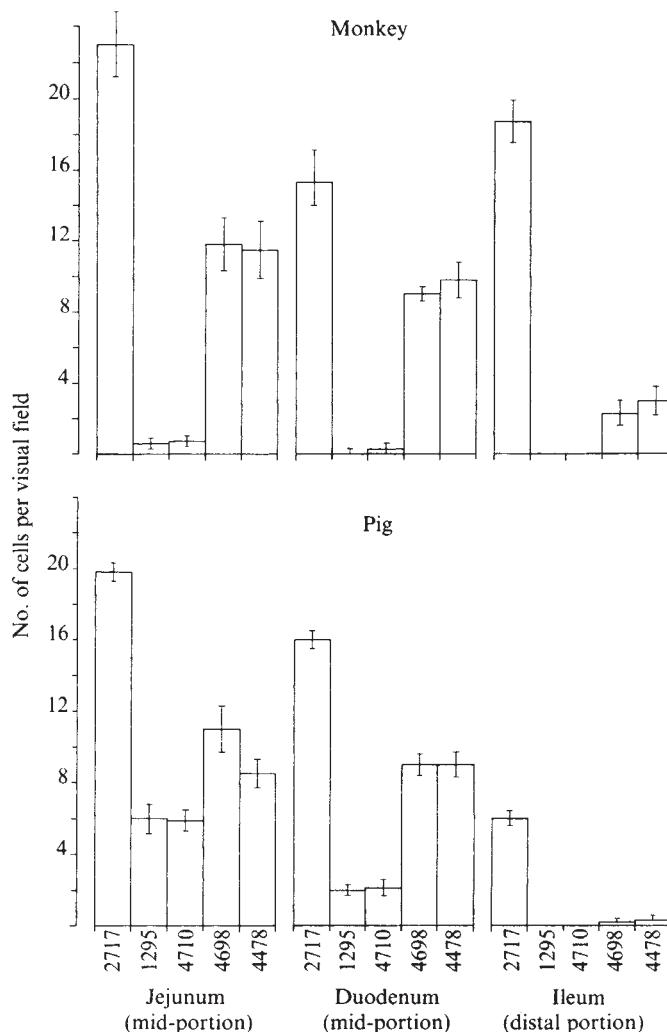


Fig. 2 Frequency distribution of intestinal endocrine cells reactive with COOH terminus-specific (Abs 2717 and 4562), CCK-specific (Abs 4478 and 4698) and gastrin-specific (Abs 1295 and 4710) antibodies. Note that in all parts of the small intestines the number of COOH terminus-reactive cells exceeds the number of gastrin and CCK cells. This difference reflects the number of TG cells. Pig ($n = 10$) material was collected at a nearby abattoir and green monkeys ($n = 5$) were anaesthetised with Mebumal (pentobarbital). Specimens from the gastrointestinal tract were frozen in melting Freon-22, freeze-dried, vapour-fixed in paraformaldehyde and embedded in paraffin. Serial 3- μ m sections were reacted with antibodies detecting the COOH terminus (Abs 2717 and 4562), the 6-13 region (Ab. 4710) and the NH₂ terminus (Ab. 1295) of human gastrin-17 as well as antibodies detecting regions 19-25 (Ab. 4478) and 25-30 (Ab. 4698) of porcine CCK-33 (ref. 1). The antisera were immunoabsorbed and then stained as described previously^{1,2}. Specificity was checked by conventional staining controls and by absorptions against synthetic human gastrin-17, the synthetic mid-portion (6-13) of human gastrin-17, pentagastrin, 99% pure porcine CCK-33, caerulein and other gut peptides^{1,2}. The site of antigen-antibody reaction was revealed by the peroxidase-antiperoxidase (PAP) procedure of Sternberger or by immunofluorescence⁷. For quantitation, serial sections were stained with the different antisera (PAP technique only), so that the first and last slides in one series were stained with the same antiserum. The variation between these two slides never exceeded, and was usually considerably below, 5%. Only perfectly transverse sections were counted and the results were evaluated as numbers of cells per field of vision (objective $\times 10$, eye-piece $\times 12.5$) or as absolute figures (data not shown). The total surface area of the (mucosal) sections averaged 0.25-0.5 cm². The number of cells was always counted in corresponding adjacent visual fields.

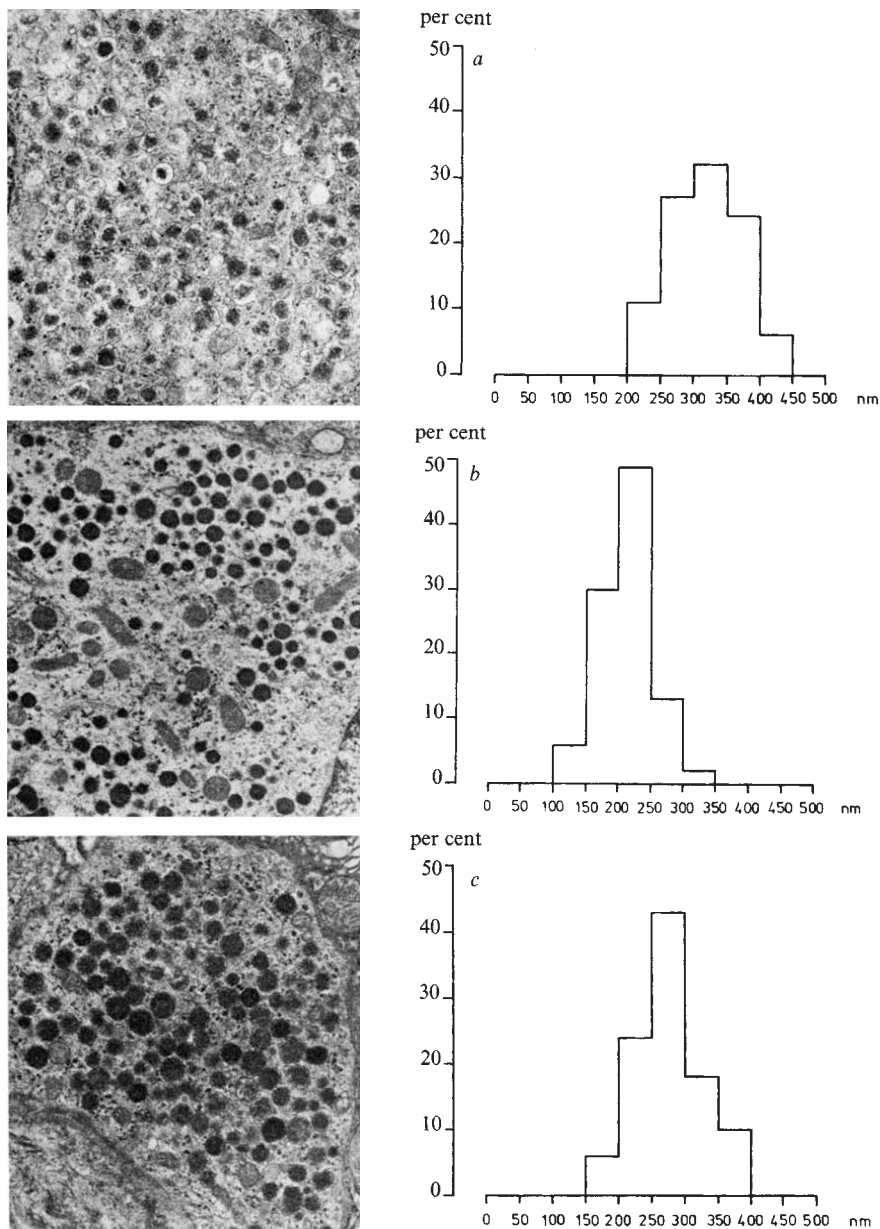


Fig. 3 Electron micrographs and size distribution histograms of cytoplasmic granules in *a*, gastrin (G) cells; *b*, CCK (I) cells; and *c*, TG cells of green monkeys. Note the differences in size and morphology of the three granule types. ($\times 7,100$). Specimens from the antropyloric and duodenal mucosa of green monkeys ($n=3$) were fixed by immersion for 15 min at 4°C in a 2% glutaraldehyde: 3% formaldehyde solution in 0.1 M sodium phosphate buffer pH 7.3. After washing in buffer and osmication the specimens were embedded in Epon 812 and adjacent semithin and ultrathin sections were cut. Semithin sections were deplasticised and de-osmicated and permitted to react with the region-specific antisera⁸ (see Fig. 1). The site of antigen-antibody reaction was revealed by immunofluorescence and immunoreactive cells were identified in the adjacent ultrathin sections. Controls were as described in Fig. 1.

shown schematically in Fig. 1. As. 1295, 4710, 4698 and 4478 contained additional populations of COOH terminus-reactive antibodies which were removed by immunoabsorption against either CCK-33 (As. 1295 and 4710) or synthetic human gastrin-17 (As. 4698 and 4478). Following the immunoabsorption step the sequence-specificity of the antibodies was verified by absorptions against synthetic human gastrin-17, the synthetic (6-13) region of human gastrin-17, pentagastrin (Peptavlon) synthetic caerulein and 99% pure porcine CCK-33 and by the inability of As. 4698 and 4478 to react with antral gastrin cells, by the inability of As. 1295 and 4710 to react with intestinal CCK cells and by the ability of As. 4562 and 2717 to demonstrate both gastrin and CCK cells, as reported elsewhere in detail^{1,2}. Our immunocytochemical results were quantitated as described in Fig. 2.

Use of gastrin-specific antisera (As. 1295 and 4710) revealed that gastrin cells predominated in the antropyloric mucosa and were few in the small intestines (Fig. 2). The oxyntic mucosa of the stomach, the Brunner glands of the duodenum and the distal ileum only occasionally contained gastrin cells (Fig. 2). The colon was devoid of such cells.

The CCK-specific antisera (As. 4698 and 4478) demonstrated numerous endocrine cells in the duodenum and jejunum. The proximal ileum contained a few scattered CCK cells, whereas the stomach, the duodenal glands of Brunner and the distal

ileum were devoid (monkeys) or nearly devoid (pigs) of such cells (Fig. 2).

The COOH terminus-specific antisera (As. 2717 and 4562) demonstrated both gastrin and CCK cells, as well as a third population of intestinal endocrine cells, which failed to react with the gastrin- or CCK-specific antisera. The existence of the third cell population was inferred from the finding that the COOH terminus-specific antisera demonstrated many cells in both the Brunner gland area and in the distal ileum. Cell counts (Fig. 2) verified the existence of the third cell type, the sum of COOH terminus-reactive cells greatly exceeding the sum of gastrin and CCK cells. Thus, COOH terminus-reactive cells, distinct from gastrin and CCK cells (TG cells), were few in the antropyloric mucosa and numerous in the small intestines. They were particularly well represented in the Brunner glands of the duodenum and in the distal ileum, areas in which gastrin and CCK cells were virtually absent (Fig. 2). TG cells were not detected in the oxyntic mucosa or in the colon.

Identification of gastrin, CCK and TG cells by electron microscopy was carried out in the monkey antropyloric and duodenal mucosa using the region-specific gastrin and CCK antibodies. Antral gastrin cells were characterised by the presence of numerous cytoplasmic granules of variable electron density. Most granules contained an irregular dense core and measured 221-442 nm (mean 307 nm, see Fig. 3). The cells

reached the lumen through an apex endowed with microvilli, and contained organelles typical of peptide- or protein-synthesising cells. This ultrastructure is similar to that of G cells in other species^{3,4}.

Duodenal CCK cells were elongated or flask-shaped and contained numerous membrane-bound granules with a thin electron-lucent halo separating the highly electron-dense core from the membrane. The granules were round or slightly angular and measured 128–383 nm (mean 209 nm, see Fig. 3). Their ultrastructure is similar to that of the I cells of the small intestines, which have been shown to produce CCK⁵.

The TG cells were identified in both the antropyloric gland area and in the duodenum. They contained large cytoplasmic granules with a core of medium to high electron density. A thin halo was usually present between the surrounding membrane and the dense core. The dense core, which almost completely filled the granules, was filamentous in texture and the granules measured 178–400 nm (mean 275 nm, see Fig. 3). The TG cells contained numerous microtubules and microfilaments and both free and membrane-bound ribosomes (Fig. 4). The ultrastructure of the TG cell is therefore quite distinct from that of gastrin-producing G cells and CCK-producing I cells, especially with respect to the morphology of the cytoplasmic granules.

As the distal half of the ileum of both pigs and monkeys contained many TG cells but very few gastrin and CCK cells, this part of the intestine was extracted in boiling water and/or acetic acid for radioimmunoassay⁶. As expected from immunocytochemical findings, only very low levels of immunoreactivity could be measured with gastrin-specific (As. 2604) and CCK-specific (As. 4698)⁶ assays. Larger amounts of immunoreactivity were, however, detected by a COOH terminus-specific assay with As. 2609⁶. Gel chromatography of ileal extracts, monitored by CCK-, gastrin- and COOH-terminal-specific assays revealed low concentrations of immunoreactivity in positions corresponding to CCK-8, gastrin-34 and gastrin component I. Gastrin-17-like immunoreactivity was not detectable. In contrast, considerable amounts of immunoreactivity eluted in the position corresponding to the COOH-terminal tetrapeptide amide of gastrin (Fig. 5). This component has not yet been shown to be identical with the true COOH-terminal tetrapeptide amide, but in parallel studies on the porcine antropyloric mucosa (to be published elsewhere) strong evidence for the existence of this component was obtained.

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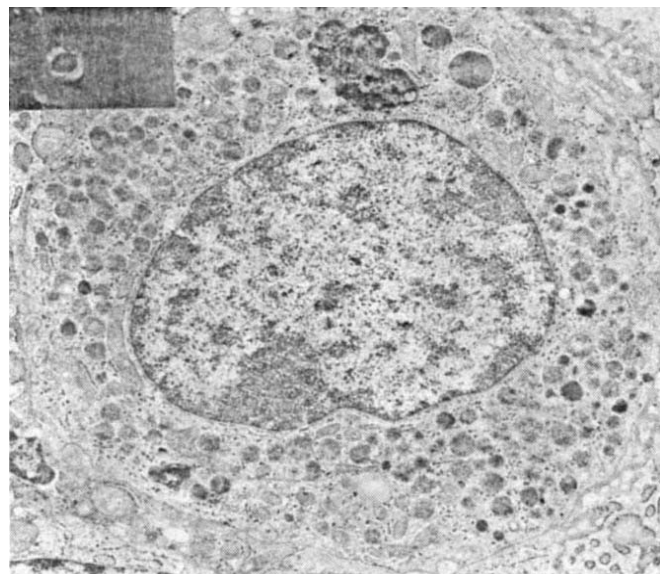


Fig. 4 Ultrastructural identification of TG cell of the Brunner gland area of monkey duodenum. The immunoreactive cell (insert: $\times 422$) contains cytoplasmic granules of variable electron-density. Technique as described in Fig. 2. $\times 4,875$.

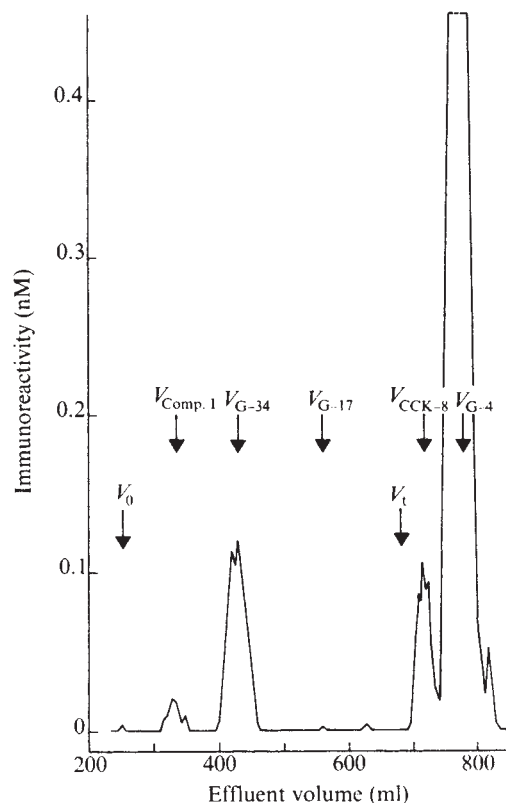


Fig. 5 Gel chromatography of a boiling-water extract of mucosa from the midsegment of the porcine ileum. 6 ml extract was applied to a Sephadex G-50 superfine column ($25 \times 2,000$ mm) eluted at a flow rate of 20 ml h^{-1} with $0.25 \text{ M NH}_4\text{HCO}_3$, pH 8.2 at 4°C . The column was calibrated with ^{125}I -albumin (void volume (V_0)), highly purified porcine gastrin component I ($V_{\text{Comp.1}}$), gastrin-34 (V_{G34}), gastrin-17 (V_{G17}), CCK-8 (V_{CCK8}), highly purified synthetic gastrin tetrapeptide amide (V_{G4}) and $^{22}\text{NaCl}$ (total volume (V_t)). In addition to boiling water, midsegments of porcine ileum were also extracted ($0.1\text{--}0.2 \text{ g mucosa ml}^{-1}$) with 0.5 M acetic acid, acetone (Merck), and acetone acidified with 0.1 M HCl ($100:3$, pH 1.0). However, when measured with an assay specific for the common COOH-terminal sequence of gastrin and CCK (antiserum 2609, also used to monitor the gel chromatography as shown) the latter procedures extracted $<8\%$ of the immunoreactivity measured in boiling-water extracts. Using boiling water extraction the following concentrations of immunoreactive gastrin-CCK-like peptides were found in mucosa from mid-ileum: Component I, ~ 0.2 ; gastrin-34, 2.3 ± 1.1 ; gastrin-17, <0.2 ; CCK-8, 14 ± 6 ; gastrin-4, 113 ± 7 (pmol per g mucosa wet weight; mean $\pm$ s.e.m., $n = 3$).

microscope facilities, Mrs J. B. Lauridsen and Mrs E. Petersen for technical assistance and Drs J. H. Walsh, J. Morley and Professor V. Mutt for antibody 1295 and natural and synthetic peptides.

Note added in proof: Recently, Buchan *et al.*⁹ described the ultrastructure of the intestinal gastrin cell. These small-granulated, D_1 -like cells seem to be more numerous in the human than in the porcine or simian small intestines. Their ultrastructure is similar to that of small-granulated, gastrin immunoreactive cells described by us in human fetal duodenum¹⁰ and in rat antropyloric mucosa (G_a cells¹¹). Thus, the small intestines harbour three types of cells producing related peptide hormones: CCK (I) cells, COOH-terminal tetrapeptide amide (TG) cells and intestinal gastrin (GI) cells. The main cell type in the antrum is the G (gastrin) cell, but some species in addition contain small numbers of TG cells (monkeys) and G_a cells. The G_a cell may possibly be the murine equivalent of GI cells.

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Protein structural fluctuations during a period of 100 ps

A RECENT 9-ps molecular dynamics simulation¹ of the bovine pancreatic trypsin inhibitor (PTI) at 295 K revealed a rich variety of motional phenomena at the atomic level on a picosecond time scale. To obtain information about longer time processes, and to characterise more accurately the short time results, a 96-ps dynamical simulation of PTI at an average temperature of 306 K has been completed with the techniques used previously¹; an extended equilibration period of 72 ps before simulation served to eliminate internal stresses. Analysis of the present simulation has confirmed most of the conclusions of the earlier study but has shown in addition that there are significant features of the dynamics that can be observed only over a longer period, ~100 ps.

An overall measure of the correspondence between the time-average dynamical and X-ray structures² is given by the radius of gyration. It equals 10.22 Å (with fluctuations of ± 0.1 Å) for the former and 10.96 Å for the latter. The primary source of difference between the two are the positions of the charged external side chains, which may in fact have different average positions in solution and in the crystal^{3,4}. The dynamics simulation also shows a strong coupling between the amino and carboxyl termini, in agreement with NMR solution results⁵ but different from the crystal structure (see Fig. 1 of ref. 1). The core of the protein has a heavy-atom number density close to the X-ray value; within a radius of 8 Å from the protein centre, the calculated number density is 0.063 Å^{-3} , compared with 0.060 Å^{-3} for the X-ray structure.

Analysis of individual atom position fluctuations shows that, as a result of the more careful equilibration, they are slightly smaller than in the previous simulation. The average r.m.s. fluctuation for the α carbons is 0.60 Å (previously 0.74 Å) and that for all atoms 0.75 Å (previously 0.90 Å); the corresponding X-ray results from isotropic temperature factors are 0.68 Å and 0.74 Å, respectively (J. Deisenhofer, personal communication). The calculated mean-square positional fluctuations are found to be highly anisotropic for most of the atoms. For the α carbons, the overall averages are $\langle z^2 \rangle = 0.252 \text{ Å}^2$, $\langle x^2 \rangle = 0.051 \text{ Å}^2$ and $\langle y^2 \rangle = 0.099 \text{ Å}^2$, where a local principal axis system is used for each atom; in some cases, the largest component is more than 10 times the smallest. The differences in the orientations of the local systems for atoms in the same region, relative to a space-fixed axis system, make clear that the anisotropy is reflecting structural constraints rather than overall protein motion. Evaluation of the positional distribution functions shows that many of them differ significantly from the standard gaussian form, a consequence of the fact that the potential of mean force experienced by the atoms is not harmonic.

Because of their well-defined nature and possibility for direct experimental investigation, the aromatic side chain motions in PTI were studied in detail⁶. Tyrosine ring orientation fluctuations of $\pm 30^\circ$ from the average (r.m.s. value of 12°) were found for residues in the interior of the protein; this corresponds to a torsional potential of mean force considerably softer than that of the rigid protein. The time-dependence of the torsional motion approximates that expected from a Langevin equation for an

angular oscillator with near critical damping; the calculated relaxation times for this motion are of the order of tenths of ps; for example, for Tyr 21 it is 0.2 ps. Comparison between the tyrosine side chain in a dipeptide and in the protein clearly demonstrates the importance of matrix damping effects due to the surrounding atoms in the latter.

An important result of the new simulation is the presence of long-lived fluctuations in the properties of PTI. Making use of 1-ps dynamic averages ('coarse-graining') to damp out rapid oscillations, it was found that fluctuations of $5\text{--}15 \text{ kcal mol}^{-1}$ with a duration of 2–15-ps occur in the potential energy associated with the bond-stretching and bond-angle-bending coordinates, as well as with the non-bonded interactions. It seems that these energy fluctuations represent transitions of the protein from one potential minimum to another in the neighbourhood of the time-averaged structure. Direct evidence for this is given by the correlation of the potential energy fluctuations with transitions between minima of side chain dihedral angles and other internal coordinates.

A consequence of the infrequent structural transitions is that long periods of averaging are required to establish the fluctuations in global properties. Using the entire 96-ps period, we find the r.m.s. fluctuation in the kinetic energy to be $11.51 \pm 0.1 \text{ kcal mol}^{-1}$, which yields a classical heat capacity of $3.14 \pm 0.1k$ per protein atom (k is the Boltzmann constant). The previous simulation resulted in a smaller value for the r.m.s. kinetic energy fluctuation ($9.3 \text{ kcal mol}^{-1}$) and a correspondingly smaller heat capacity ($2.3k$ per atom); test calculations show that such low values are due to the short time interval used for averaging. Apparently the heat capacity contains a contribution from equilibration among the different available states and so cannot be related as simply to models for protein dynamics as assumed in ref. 1. Long-lived fluctuations also occur in the atom positions, so that values obtained by averaging over less than 10 ps tend to be too low; for example, for the α carbon atoms, the r.m.s. fluctuation from 2-ps intervals are ~ 0.40 Å as compared with the value of 0.60 Å for the complete simulation.

The present calculation supports the earlier conclusion that a dynamic simulation can provide realistic and highly informative descriptions of the detailed atomic motions in the interior of a protein. The existence of large fluctuations in the structure and energy components indicates that the properties of proteins result from the occupation of a variety of thermally accessible states, even for times as short as 10–100 ps. It will be of interest to assess the role of these states in the biological activity of proteins and to determine the importance of alterations in the available states and transitions among them due to the solvent environment and ligand or substrate binding.

A full account of the extended simulation will be presented elsewhere. We thank John Ramsdell, Barry Olafson, and Thomas Jordan for their assistance and the NIH for support. J. A. McC. thanks the NIH for a postdoctoral fellowship.

Note added in proof: Frauenfelder, Petsko, and Tsernoglou (personal communication) have recently determined isotropic temperature factors for myoglobin at a series of temperatures and have used them to obtain atomic positional fluctuations; a dynamical simulation to compare with their results is in progress.

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reviews

Some pioneers of Natural History

Ray Desmond

The Naturalists: Pioneers of Natural History. By A. C. Jenkins. Pp. 200. (Hamish Hamilton: London, 1978.) £6.95.

MAN has always had some interest in animals and plants, if for no other reason than that they provide him with food. But curiosity rather than necessity compelled the attention of people like Aristotle who was one of the earliest scientific observers of nature. His *Historia animalium* shaped and directed scientific thought and enquiry for centuries.

Mr Jenkins's survey of the lives and achievements of some of the pioneers of Natural History begins with Aristotle and Pliny, and he pauses to consider mediaeval bestiaries and herbals before introducing the first encyclopaedic systematist, John Ray. Ray's skill and industry in classifying animals and plants earned him the appellation, "the Aristotle of England". But he was to be overshadowed by the Swedish naturalist, Carl Linnaeus, as the supreme classifier of the many forms of life. Linnaeus's sexual classification of plants, based upon the number of stamens a flower possessed, provided the professional and amateur botanist with a delightfully easy method of identifying plants, although one naturalist, Johann Siegesbäck of St Petersburg, rejected it as a "lewd and licentious system".

Both great and lowly have been devotees of the study of Nature. Mr Jenkins records that Frederick II, King of Sicily and Emperor of Germany, became a passionate naturalist through falconry and created a number of zoological gardens. He could also have mentioned the Moghul Emperor, Babur, who even during his conquest of India had time to observe a handsome plant or an unfamiliar animal.

Natural History has always provided a useful role for the amateur. Sir Joseph Banks, perhaps the most influential of all amateur naturalists, discovered its delights while a student at Oxford and after graduating persuaded his mother to rent a house at Chelsea so as to be near the Physic Garden of the Society of Apothecaries. It is said he paid £10,000 for the privilege of accompanying Captain Cook as naturalist on his first circumnavigation of the world. He encouraged Sir James

Edward Smith, another enthusiastic amateur, to buy Linnaeus's collection of plants, insects, shells, minerals and manuscripts. He despatched gardeners to many parts of the known world to collect new plants for the King's gardens at Kew and Windsor.

Plant collectors were often explorers in their own right, travelling through unknown lands and risking their lives at the hands of hostile natives. Mr Jenkins praises the fortitude of David Douglas, a gardener at Glasgow Botanic Gardens, who was sent to North America in 1823 by the Horticultural Society of London to collect plants. Always ill at ease in society, Douglas was happiest on his own in the wilds of Canada where he endured incredible hardships in his pursuit of plants. The observation of Nature meant everything to him. There was the occasion when, despite extreme hunger, he would not allow a Franklin's goose which had been shot to be eaten because he wanted it for scientific purposes. He was only 35 when he was trampled to death by an enraged bull in an animal pit in Hawaii.

Not all naturalists led such adventurous lives. The French entomologist, Jean Henri Fabre, never left his native Provence, and the Reverend Gilbert White found fulfilment in his acute observation of natural things at Selborne in Hampshire. Indeed many clergymen escaped from the tedium of parochial duties through a study of botany, ornithology, entomology or some other branch of Natural History.

Natural History has its share of eccentrics and Charles Waterton, squire of Walton Hall in Yorkshire, is one of the most endearing of them all. He spent ten years building a high wall around his estate to protect wildlife from foxes, badgers and poachers. In his *Wanderings in South America*, he describes his vain attempts to persuade the vampire bat to suck his blood, and his bare-back ride on an alligator is a classic anecdote. As a devout Catholic he could not resist using his considerable skills in taxidermy to create animal caricatures of leading Protestants. He ridiculed Darwin's evolutionary theory by fashioning a simian-like creature from the skin of a red howler monkey and solemnly announcing it was

the missing link in man's evolutionary chain.

Of course Charles Darwin had to be given a chapter in this book but the author spares a few pages for his fellow evolutionist, Alfred Russel Wallace, who meekly, almost masochistically, allowed himself to be eclipsed by Darwin.

Towards the end of his work Mr Jenkins admits that it "would more accurately be subtitled 'Some pioneers of Natural History' presenting as it does no more than a handful of naturalists". It is an easy matter to point to omissions. The two great bird painters, Audubon and Gould, are included but there is no mention of any eminent botanical artists such as Ehret and Redouté. In fact the zoological interests of the author predominate. Much of the author's life has been devoted to the cause of animal conservation and he informs us rather sadly that even Audubon indulged in the wanton killing of birds. He is an active supporter of zoos and discusses their role in protecting endangered species.

Mr Jenkins writes with enthusiasm and conviction but not always with accuracy. There is a sprinkling of mistakes, some of them clearly the result of careless proof-reading. The Linnean Society was founded in 1788 not 1778 (p46); William Harvey died in 1657 not 1677 (p192); the drawing of a lupin on p96 comes from the *Botanical Register* and not the *Botanic* (sic) *Magazine*; George III's mother, Princess Augusta, was never Queen (p88). Why are only floruit dates given for Nicholas Culpeper, John Gerard and William Turner (pp191-93) when the dates of birth or death are known?

Compared with Joseph Kastner's *A World of Naturalists* (John Murray: London, 1978) and David Allen's *Naturalist in Britain: a Social History* (Allen Lane: London, 1976), Mr Jenkins's account is lightweight indeed. However, with its generous and well chosen selection of plates, some in colour, it can be recommended as a pleasant introduction to the subject. □

Ray Desmond is Deputy Keeper at the India Office Library and Records, and a Past President of the Society for the Bibliography of Natural History.

Assessment of medical technology

The Image and the Reality: A Case Study of the Impacts of Medical Technology. By B. Stocking and S. L. Morrison. Pp. 80. (Oxford University Press: Oxford, London and New York, 1978.) Paperback £3.

THERE is no doubt about the public appeal of medical technology, as the achievement of a remarkable lady in Lancashire is raising nearly £1.5 million to buy and run a body scanner attests. Yet as the Director-General of WHO has recently said: "Technology for the sake of technology is a dangerous addiction-producing drug". Stocking and Morrison tackle the problems of technology assessment taking the body scanner as a case study.

The development of computed tomographic (CT) scanning of the brain by EMI, funded by the Department of Health and Social Security, was a remarkable British success story leading to clear improvements in diagnosis. The same technique—X-rays passed through the body, detected by scintillation counters coupled to a computer—was then extended to whole body scanning. Whereas clinical evaluation had accompanied development of the brain scanner, in the case of whole body scanners evaluation was more complex because of the variety of organs involved and of alternative diagnostic techniques and they were extensively introduced before there had been thorough clinical evaluation. The most rapid diffusion of body scanners has taken place in the USA (700 machines by 1977) and is largely attributable to their profitability, whereas in Britain diffusion has been much slower (11 machines by 1977) and for most of these the capital, but not running, costs have been met by charities rather than the health service.

The impact of body scanners is considered in this book on three broad levels: for patients, in terms of diagnosis, patient management and outcome; for the health service, in terms of the pattern and demand for care and for the professions involved; and for industry involved in manufacturing the equipment. As assessment of any technology must be concerned with the future, the authors discuss a variety of changes that may affect the contribution of CT scanners such as future developments of CT scanners, advances in other imaging techniques and in therapy, changes in cost and health service expenditure. This of

course introduces many uncertainties but it is surely wise to try and see what may develop and the implications; as decisions affect the future it makes little sense to base them solely on an evaluation of the past.

In assessing medical technology, what is ultimately most important is the effect on outcome for the patient. Randomised controlled trials are common, if far from universal, practice in evaluating new drugs and therapies—however unusual such trials may be in assessing accepted techniques and therapies—and there is every reason to extend them to diagnostic procedures. Yet this is only just beginning.

Stocking and Morrison write: "The remarkable diffusion of [whole body scanners] prior to proper clinical evaluation is unsatisfactory". This is perhaps an understatement. The image of the health service promoted by the medical profession in Britain and many other countries is that it is crumbling from lack of resources and deprived of new developments that could be saving lives. What may be closer to the reality is that much of the medical profession is quite unscientific, indifferent to clinical evaluation and infatuated with technology and that there are ample resources if only they can be

re-directed from activities which are useless and downright harmful into those where they may do some good.

This slim volume is interesting as a case study, and one can only lament that it is the first such study to approach a new technology in a systematic and comprehensive way. It is still more valuable in that it poses fundamental questions about the nature of health services and the impact of technology on these—which is not to doubt the highly beneficial effects in many cases. Finally, the authors make a positive proposal for a Medical Technology Assessment Group. With the prospect of almost limitless extensions of what *can* be done with medical technology, decisions as to what *should* be done can only become more pressing. The sooner Departments of Health and the World Health Organisation regard the assessment of medical technology as central to their functions the more hope there is of shaping health services to conform with the needs of human life.

David Piachaud

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Testicular function

The Mammalian Testis. By R. B. Setchell. Pp. 450. (Paul Elek: London, 1978.) £18.

THE testis is responsible for the production of spermatozoa and testicular hormones. Highlights of spermatozoa production include the initial mitotic divisions, the setting aside of stem cells which will re-initiate the spermatogenic process at some later date, meiosis, and finally, the dramatic metamorphosis of the prospective gamete that results in the formation of the acrosome, flagellum and mitochondrial helix and in the condensation of nuclear chromatin. Interestingly, many of these events occur only if the prospective gamete is sequestered behind the blood-testis barrier formed by the Sertoli-Sertoli occluding junctions bathed in fluids rich in steroid hormones and protected from noxious stimuli such as elevated temperature and radiation.

Dr Setchell's book deals with all the above aspects of testicular function. This is a formidable task because it requires that the author be knowledgeable in anatomy, physiology, bio-

chemistry and endocrinology. For the most part, Dr Setchell achieves this difficult task with the result that the book is informative, clearly written, well illustrated and completely referenced. It will provide a valuable source of information for all those interested in testicular structure and function.

The chapters on vascular and nervous supply of the testis and scrotum, on fluid secretion and entry of substances into the seminiferous tubules, and on testicular thermoregulation are extremely good and reflect the author's long-standing research interest and expertise on these subjects. The chapters on spermatogenesis, endocrinology of the testis and endocrine control of the testis are presented in a thorough and professional manner. The chapters dealing with testicular metabolism and naturally occurring and induced dysfunctions of the testis are informative but not insightful. They consist primarily of a recitation of literature references and contain little or no synthesis of concepts. Overall, this is an excellent book and Dr Setchell should be congratulated.

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Cooperative equilibria

Cooperative Equilibria in Physical Biochemistry. By D. Poland. Pp. 344. (Clarendon/Oxford University Press: Oxford, 1978.) £14.

COOPERATIVITY is an important phenomenon in molecular biology. It gives rise to sigmoidal binding curves, to allosterism, to steep conformational transitions, and plays a major role in assembly processes. Quantitative treatments were developed for many simple systems and a general understanding was obtained for more complex ones. So far, the only summaries available were contained in several review articles and in a rather specialised book by Poland and Scheraga (*Theory of Helix Coil Transition in Biopolymers*, Academic, 1970).

The present book takes a more general approach. It starts from basic principles, gives a simplified presentation of the equilibrium theory and treats some specific systems as examples for its application. The book originated from a series of courses presented by the author to students at Johns Hopkins University at various levels of their curriculum. It has the nature of a lecture script, is well written and reflects considerable teaching experience. The first chapter on simple multiple equilibria is rather elementary. In a long second chapter some concepts of statistical mechanics are repeated and some basic physical principles of intermolecular forces are presented. Three more specific and more demanding chapters are on transitions in linear biopolymers composed of single and multiple chains and on cooperative binding of ligands to linear macromolecules and to a two-dimensional lattice. A last chapter deals with the complications which are introduced when macromolecules are not composed of identical, repeating units but have a more complex sequence.

The main advantage of the book is that it may serve as a well written text for course work or for self-study. It lacks, however, a treatment of cooperativity in self assembly, a topic which is rather important and straightforward for linear systems like actin filaments or bacterial flagella. Two- or three-dimensional problems like the folding of globular proteins or transitions in membranes are not treated in any detail. Most importantly, I feel that a course on cooperative phenomena will be incomplete if the important kinetic features of cooperative systems are left out completely. My own teaching experience shows that equilibria and

kinetics can be presented together with considerable advantage for a deeper understanding.

The author did not intend to present a systematic coverage of the research in the field of cooperative systems. The usefulness of the book as a review and as a source of references is therefore limited. References to the literature are few in number and incomplete. A rather severe disadvantage is the lack of a subject index. It

has to be emphasised, however, that a short monograph like this can never satisfy all wishes and that the book will certainly be welcomed by many students and research workers who wish to be introduced into the field of cooperative equilibria.

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Moorlands and montane grasslands

Production Ecology of British Moors and Montane Grasslands. Edited by O. W. Heal, D. F. Perkins and W. M. Brown. Pp. 426. (Springer: Berlin, Heidelberg and New York, 1978.) DM108; \$54.

THIS book comprises a series of research reports from the British part of the International Biological Programme (IBP) studies, mainly from Moor House in the northern Pennines and the sheep-grazed montane grasslands of Snowdonia. The overall emphasis is upon the development of ecosystem models for the two habitats based upon a variety of experimental studies of the component parts, together with mathematical models and simulations of certain critical processes, such as production, decomposition and peat accretion.

Much of the data from the Moor House IBP study has been published elsewhere, though it is useful to have much scattered data collated and condensed here. The field studies of Smith and Forrest and the controlled environment work of Grace and Marks on the primary productivity of the blanket mire ecosystem have provided an interesting set of data from a generally neglected habitat. It is particularly useful to have in the same volume some comparable data from the southern English heaths (Chapman and Webb) and from the Cairngorm dwarf heath communities (Summers). Mean production values for the Moor House *Calluna/Eriophorum* blanket mire sites are about $250 \text{ gm}^{-2} \text{ yr}^{-1}$, those from Dorset heaths about $320 \text{ gm}^{-2} \text{ yr}^{-1}$, and from the Cairngorms come figures which vary considerably with such factors as soil fertility and degree of exposure, from $75\text{--}300 \text{ gm}^{-2} \text{ yr}^{-1}$. Given the generally small sample areas studied, these figures are in very satisfactory agreement.

One advantage of publishing a collection of site orientated papers is the opportunity of putting together work which would otherwise be published in widely scattered disciplinary journals. So it is valuable to have the Moor House work on fauna and microbes in the same volume as the primary productivity work. A paper which will prove of particular value to peatland ecologists in general is that by Martin and Holding on the factors influencing microbial activity in blanket peat. Microbial activity was stimulated by adding an organic carbon source or by adding nitrogen, indicating nutrient limitations. The physical structure of the peat may restrict access to organic reserves. From the production and decomposition data, Jones and Gore construct a simulation model and Clymo develops a model of bog growth.

The Welsh grassland work is similar to that at Moor House in that it deals with a montane habitat, but it differs in many other respects, such as the density of sheep. On Pennine blanket mire this was only 0.13 per hectare, whereas on the *Agrostis/Festuca* grasslands in Snowdonia it attained 9 per hectare. So the studies of sheep populations (Dale and Hughes) and their productivity (Brasher and Perkins) form important constituents of the Snowdonian work. The final summary of energy and nutrient flow patterns by Perkins is particularly useful and could have been emulated with profit by the Moor House team. From the diagram produced one can discern at a glance some fascinating aspects of the sheep-grazed ecosystem, such as the fact that there is more potassium in sheep dung (per unit area) than in sheep, and that earthworm nitrogen exceeds that in sheep whereas sheep have twice the energy content.

The bibliography is extensive and the book will prove an essential reference work to all who conduct research into these montane environments in Britain.

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Thin film devices

Active and Passive Thin Film Devices. Edited by T. J. Coutts. Pp. 858. (Academic: New York, London and San Francisco, 1978.) £60.

If one expects the value of a technical work to keep pace with its cost and size then at a price of £60 at a weight of over 3 lb a purchaser is likely to be disappointed with the work under review. Unfortunately the subject matter is neither sufficiently thorough in its range nor adequately coordinated to constitute a satisfactory reference text on the chosen theme. How, you may ask, is this possible with 858 pages at the editor's disposal: the answer is that the editor has not adopted a policy towards his reader when choosing the subject matter. This is an applied physicist's work not an engineer's, and equal emphasis is given to realised devices as well as promising ones and failures, which might be acceptable if the reader were warned accordingly at the outset.

The book, containing thirteen chapters by specialists, covers thin film preparation, electrical properties of film materials and their uses or potential uses as passive elements and active components. The chapters describing solar cells, optical waveguides and magnetic and superconducting devices are useful reviews, but the references and appended notes indicate that the book was written by 1976. Nearly two years in printing is too long for such a rapidly moving field; consequently molecular beam epitaxy is given a proof reference and solar thermal converters which are in advanced development receive three lines of text.

The development history of thin film systems is littered with failures in deposition techniques and film materials, but such is the speed with which novel devices are invented that the search for new materials and growth processes continues unabated (except perhaps in the UK). Under such circumstances what should an editor include in a book on thin film devices? First, he must decide to whom the collected reviews are directed: the dust-cover suggests physicists, electronic and production engineers and material scientists. This is a broad readership that one cannot expect to have the discrimination of specialists when judging which devices are of practical value and which of historical interest. Thus the editor has given the same emphasis to thin film transistors and threshold switches (although their effective use after a decade is still awaited) as to resistors, capacitors, photoconductors, and so on. Of course one does not want to waste knowledge of physical character-

istics arising from an unfulfilled product development but much of this could have been discussed in the chapters devoted to electrical properties of film materials. In view of the importance of silicon technology the most serious weakness perhaps is the omission of a chapter solely devoted to the growth and uses of epitaxial and amorphous silicon and of silicon compound films. Also there is an artificiality in restricting discussion to wholly thin film systems when thin films are so vital a part of most solid-state devices.

When discussing the history of the use of thin films in electronics T. J. Coutts states that there would have been no advantage of using thin film resistors in the period of bulky valve circuitry, but the requirements of microminiaturisation made their use essential. This is an incomplete view of what occurred. Thin film resistors using NiCr alloys came into use in World War II for waveguide attenuators and were later used for precision resistors. The reason why vacuum deposited films were not used in valve circuits was that they broke down when operated at voltages in the 100V region. However, development continued in the 1950s on transparent conducting coatings for windows and on the deposition of insulating films and the metallising of capacitor foils (which commenced before the War). Thus, considerable know-how existed

on passive devices when the transistor became a production item and a more correct view is the convergence of technologies resulting in the manufacture of hybrid and integrated circuits.

In the chapter on "Preparation Methods for Thin Film Devices" the author has chosen to describe a variety of techniques rather than concentrate on specific kinds favoured for making the devices considered in the other chapters. The emphasis given to metal filaments and boats for evaporation suggests a subject treatment one would have found a decade or more ago and the availability of such refractory materials as B/Ti/N complexes for the high rate evaporation of aluminium is not mentioned. Also magnetron sputtering is not described although it has helped to cause a renaissance in the use of sputtering in solid-state technology and most of the advances leading to cylindrical and planar magnetrons were made by 1974, that is, before the book was written.

The book is not an embracing text, and is inadequate in its treatment of deposition processes and lacks balance in its choice of devices. However, as stated above, there are several specialist chapters which are valuable reviews.

L. Holland

L. Holland is Head of the Unit for Plasma Materials Processing at the University of Sussex, UK.

Reflection seismology

Reflection Seismology: A Tool for Energy Resource Exploration. By K. H. Waters. Pp. 377. (Wiley: New York and Chichester, UK, 1978.) £19.75.

By far the most successful method of determining the structure of the upper few kilometres of the Earth's crust has been and continues to be the art and science of reflection seismology. This book provides an essentially complete survey of the subject from the basic physical principles to the multitude of data generation and analysis tools currently used by the oil exploration industry. The volume is perhaps too short on mathematics. However, the physics, including the underlying assumptions of each technique, are generally well presented.

The volume begins with a general description of the pertinent aspects of elastic wave propagation in the upper crust of the Earth. Following chapters cover seismic energy generation techniques, data-gathering methods, stack-

ing and filtering techniques, and the reduction of processed data to the geometry and physical properties of the rock formations below. Introductions to such recent and current topics as homomorphic deconvolution, adaptive filtering, maximum entropy spectral analysis, integral and differential wave equation migration, shear waves, high frequency methods and three-dimensional data collection and analysis are included.

The professional exploration seismologist will find that they know a great deal more about many of the subjects covered in this book, and students whose desire is a thorough knowledge of any given topic will require a more detailed and explanatory presentation. However, the volume is well referenced (up to about 1973). The book is appropriate and recommended for those who want a basic technical introduction to exploration seismology from beginning to end.

Tom Landers

Tom Landers is a staff member at the Applied Seismology Group of Lincoln Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts.

obituary

Norman Feather, 1904-1978

PROFESSOR NORMAN FEATHER, FRS, died on 14 August 1978 at the age of 73. His death has removed one of the last of that brilliant group of young men who gathered round Rutherford at the Cavendish Laboratory, and later guided the postwar expansion and flowering of academic physics in Britain.

Feather was born at Crimsworth, in the West Riding, the son of two school teachers. At Bridlington School he came under the influence of an inspiring science master, and this led him to decide to study physics at Trinity College, Cambridge. In 1926 Feather was awarded his BA degree at Cambridge, and the BSc degree of the University of London.

For the next three years he worked as a research student, under Chadwick, on the development of cloud-chamber techniques and their application to a study of the rare long-range α -particles from Radium-C. In 1929 he was awarded a Fellowship of Trinity College. The award was for a period of four years, and he spent the first of these years at Johns Hopkins University in Baltimore, at the behest of R. W. Wood. He returned to Trinity College in 1930 and submitted—successfully—his PhD thesis.

In his luggage Feather had brought back with him 300 'dead' radon bulbs, from the Kelly Hospital in Baltimore, containing in all about 100 millicuries of polonium. It was with this polonium that Chadwick, Dee, and Feather established in 1932 the production of neutrons when beryllium was bombarded by α -particles, and in particular Feather, using his automatic cloud-chamber, produced the first clear evidence for the transmutation of nuclei by neutrons.

Feather's next cloud-chamber experiment was a study, jointly with Bretscher, of the photo-disintegration of the deuteron. The value they obtained for the deuteron binding energy was challenged by Bethe, on the basis of his theoretical studies of the rate of energy loss of charged particles in matter. The Bretscher-Feather value was subsequently vindicated by more refined experiments elsewhere. Feather had never doubted that it would be: his calibration data had been accumulated during years of painstaking observation and comparison.

Feather frequently pointed out to his colleagues and juniors that he had

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never been exposed to the higher reaches of mathematics nor to any quantum theory—'just a little applied mathematics'. And he would go on to maintain, with a glint in his eye, that there were still things to do in nuclear physics where insight and intuition were the best guides. When it was necessary his mathematics tended to rise to the occasion, as in his early studies of the statistics of the prompt- and delayed-coincidence techniques which he used in β -ray spectrometry and the investigation of nuclear isomerism, in the late 1930s. But when his insight and intuition were involved they were backed up by a vast and detailed knowledge of nuclear systematics, which found expression in his monograph on *Nuclear Stability Rules* (1952) and in his later writings on nuclear fission.

When Chadwick went to the Chair of Physics in Liverpool in 1935 Feather went with him, but remained there for only one year, during which he wrote his first undergraduate textbook, *An Introduction to Nuclear Physics*. In 1936 he returned to a lectureship in Cambridge, in charge of his own research group. A year later Rutherford died, and subsequently Feather found himself with more responsibility for the nuclear work in the Cavendish Laboratory. During the war years he carried a very heavy administrative load, both in the teaching programme and in the war-time work on nuclear fission.

In 1945 Feather was elected to the Fellowship of the Royal Society, and appointed to the Chair of Natural Philosophy in Edinburgh. He occupied this chair, as Head of Department, for 30 years. His first task in Edinburgh was to build up a department in which the techniques which had transformed nuclear experimentation during the war years could be developed and exploited. He was joined by a number of talented young men, and work was soon under way in the β and γ -ray spectrometry of (mainly) naturally radioactive species, and in radiation chemistry and fast-neutron physics. Feather was not a man to stifle or constrain those of his staff who wanted to work in other fields, and projects on cosmic rays, meteorology, fluid dynamics and metal physics got under way too. The undergraduate curriculum was drastically overhauled, and by his own example Feather transmitted his view of the interdependence of undergraduate teaching, postgraduate training, and research.

With his Department running smoothly, Feather for a time concentrated his energies on writing and produced a series of texts aimed at undergraduates. Although his rather mannered style was not altogether congenial to the undergraduates of the 1950s and 1960s, the combination in these books of care for language, extensive knowledge, and a scholarly and disciplined approach, was an affirmation of values which the author felt impelled to exhibit to his students.

In the 1960s Feather's attention turned again to the fission process, and a series of papers followed which dealt with the process of ternary fission, and especially the α -particles emitted in fission. His lifelong interest in the various aspects of α -emission continued into his retirement. His penultimate paper, published in 1978, discussed the two solutions that have been proposed to the problem of the polonium haloes in mica. Something of the essence of the man flashes out in a characteristic sentence: 'Because these two suggestions appear to exhaust the logical possibilities of explanation, it is tempting to admit that one of them must be basically correct, but whoever would make this admission must be fortified by credulity of a high order.'

Feather's keen and incisive mind and his remarkable detachment made him a distinguished academic administrator.

John Dewar Studios

His services to such bodies as the Scottish Universities Entrance Board, the Home Defence Scientific Organization, and the Royal Society of Edinburgh (which he served for many years, as Councillor, as General Secretary, and then as President) were generously given and widely appreciated. The University of Edinburgh acknowledged his notable services to the University community by the award of the Honorary Degree of Doctor of Laws, just a few weeks after he retired.

R. M. Sillitto

Herbert Dingle

THE well-known spectroscopist, astrophysicist, philosopher of science and critic of relativity, Herbert Dingle, died in Hull on 4 September 1978, not long after his 88th birthday.

Although of Cornish descent, Dingle was born in London, close to his beloved Surrey county cricket ground, the Oval, on 2 August, 1890. He spent most of his boyhood and youth in Plymouth, where his mother had returned on the death of her husband. After leaving school at the age of fourteen, Dingle worked for over ten years as a clerk by day and studied, as best he could, by night. In 1915 he won a Royal Scholarship for Physics at Imperial College. He graduated in 1918, having already been appointed to the staff of his department. The same year he married Alice Westacott. She predeceased him by over thirty years. He remained devoted to her memory. They had one son who became an industrial chemist, but he too died before his father.

More than three-quarters of Dingle's academic career was spent at Imperial College, where he was eventually promoted to a chair in natural philosophy in 1937. Originally, he specialised in spectroscopy under Alfred Fowler, the successor of Norman Lockyer. On Fowler's retirement in 1935, he became head of the spectroscopy section. Under Fowler, Imperial College had been in the forefront of physics, in the great days of line spectroscopy following the introduction of the Bohr atom. It was no fault of Dingle's that his time as head of the spectroscopy section coincided with a world-wide lull in the subject, prior to its revival, under the impact of nuclear physics, in the nineteen-fifties. Nevertheless, Dingle did useful work in spectrochemical analysis and pioneer research on the spectra of fluorine II and III. He wrote much on the laboratory and astronomical applications of spectroscopy.

His book *Modern Astrophysics*, published in 1924, was one of the first on that subject. He was also part author of *The Splendour of the Heavens*, published in 1923, and *The*

Life and Work of Sir Norman Lockyer, published in 1929.

From the early nineteen-twenties Dingle was actively concerned with the philosophical as well as with the practical aspects of physics. He developed a highly professional interest in the philosophy of science and as a writer in that field he became very well known, particularly in the United States, which he visited for two lengthy periods in the thirties, the second time as Lowell Lecturer at Harvard. During the prolonged absence of Sir George Thomson from Imperial College during World War II Dingle was effectively in charge of the physics department.

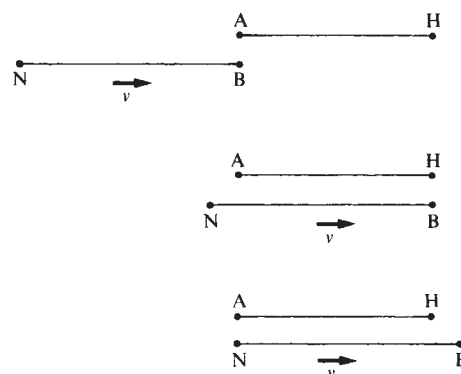
Dingle's philosophy of science, as expounded in his books *Science and Human Experience* (1931) and *Through Science to Philosophy* (1937), the latter based on his Lowell Lectures, was in the new unfashionable tradition of British empiricism. He believed that the essential basis of science was the rational correlation of elementary experiences which can be made objective. This led him to adopt a philosophy which had much in common with that of Ernst Mach.

In 1946 Dingle was appointed Professor and Head of the Department of History and Philosophy of Science at University College, London, a post that he held until his retirement in 1955. Although no longer active in spectroscopy and astrophysics, he was elected President of the Royal Astronomical Society from 1951 to 1953. He was also President of Commission 41 (History of Astronomy) of the International Astronomical Union for some years and was a Vice-President of the International Union for the History of Science from 1953 to 1956. He founded in 1948 the Philosophy of Science Group of the British Society for the History of Science (which later became the British Society for the Philosophy of Science). Two years later, with the support of the late Dr H. P. Morrison of the publishing firm Nelson, he founded *The British Journal for the Philosophy of Science*. From 1955 to 1957 he was President of the British Society for the History of Science.

In the latter year he began his famous campaign against the special theory of relativity which dominated the last twenty years of his life. He had originally been sympathetic to the theory, particularly because of the ostensibly 'operational' philosophy that had guided Einstein in its creation, and in 1940 he published a Methuen monograph on it. His doubts originated when, while reading Sir George Thomson's *The Foreseeable Future*, he came across a passage relating to the 'clock paradox'. As a result, he engaged in a controversy in the correspondence

columns of *Nature* with Professor W. H. McCrea, FRS, which led to a tremendous resurgence of interest in the 'paradox' and a spate of publications on it.

Later Dingle attacked the entire concept of relativistic time. His basic objection was that, according to the theory, two similar clocks, *A* and *B*, in uniform relative motion *V* work at different rates, but since the relation of each to the other is symmetrical it follows that, if *A* works faster than *B*, *B* must work faster than *A*. He argued that this was a contradiction and so the theory must be false. The point at issue was a subtle one and concerned the way in which Dingle used a particular concept which he called 'rate of clock'. He exemplified this by taking *A* and *B* to be together at epoch zero according to each and introducing two other clocks *H* and *N* at rest relative to *A* and *B*, respectively, but not spatially coincident with them. He



compared the times t_1 , t'_1 assigned to the epoch of *B*'s momentary coincidence with *H* by the observers using the clocks *A*, *B* respectively, and the corresponding times t_2 , t'_2 assigned to the epoch of *A*'s momentary coincidence with *N*. He then argued that, since special relativity led to the formulae, $t_1 = \beta t'_1$, $t'_2 = \beta t_2$, where β is the reciprocal of $\sqrt{1 - V^2/c^2}$, a contradiction results. This is because in the former case the ratio of the 'rates' of clocks *A* and *B* is given by t_1/t'_1 and in the latter case by t_2/t'_2 , and hence this ratio has two incompatible values.

The difficulty can be resolved by rejecting Dingle's assumption that ratios such as t_1/t'_1 determine invariant relative 'rates' of clocks *A* and *B*. His implicit requirement that the epochs assigned to any event by *A* and *B*, respectively, should always be in the same ratio would imply that by a new choice of time unit for one of these clocks it could be arranged that the times assigned to any given event by *A* and *B* would be the same. Dingle's requirement is therefore equivalent to adopting the Newtonian concept of

universal time, and this is incompatible with special relativity.

Dingle believed that his question concerning clock-rates was not answered but evaded by his critics. In his struggle with what he came to regard as the 'relativistic establishment', described in his book *Science at the Crossroads* (1972), he was often treated in a way that convinced him that truth was being sacrificed to dogma. His attitude towards relativistic physics was not, however, purely negative, for he adopted, and spent much time in developing, the alternative 'ballistic' theory of light-transmission due to W. Ritz.

Despite his criticism of special relativity, Dingle never lost his respect for Einstein's genius, just as he no less admired his fellow quaker Eddington despite submitting the latter's philosophy of physics to devastating criticism in his brilliant Eddington Memorial Lecture on *The sources of Eddington's philosophy* (1954).

No obituary notice of Dingle should fail to mention the beautiful literary style that graced all he wrote. He had a deep knowledge and appreciation of English literature, particularly poetry. Two of his books were on *Science and literary criticism* (1949) and *The mind of Emily Brontë* (1974). His friends will long remember his lively conversation spiced with a rapier-like wit and numerous anecdotes, and his many acts of kindness, not least to those refugees from Europe whom he and his wife so generously assisted in the nineteen-thirties.

G. J. Whitrow

M.H.L. Pirenne

MAURICE HENRI LÉONARD PIRENNE, well known for his work in the physiology of vision, died in Oxford on 11 October 1978.

Maurice Pirenne was born in Verviers, Belgium in 1912. He graduated DrSc at Liège, and spent the next year doing research in molecular physics with Professor Debye. The following three years were spent at Columbia University, New York, with Selig Hecht studying the biophysics of vision, and it was probably this period, more than any other, that determined his future interests.

In 1942, a classical paper by Hecht, Shlaer and Pirenne demonstrated that the uncertainty of seeing near the visual threshold, usually attributed to biological variation, was in fact largely accounted for by purely physical variations in the small number of light quanta absorbed by the visual photopigment. Much of Pirenne's later work was concerned with the visual threshold and its relationship to visual acuity, ranging in scope from the practical

problem of screening servicemen for night blindness, to neurophysiological studies of the thresholds of 'on' and 'off' units and their interaction.

He returned to England in 1941 and worked in Cambridge, London and Aberdeen before joining the University Laboratory of Physiology, Oxford, in 1955. He became a fellow of Wolfson College, and worked in Oxford until his retirement. As a teacher, he will be remembered particularly for his demonstrations and class work. The experiments that brought the subject so vividly to life were based upon long hours of preparation and adjustment.

Maurice Pirenne was the son of an artist, and while he was still at school his father encouraged him to read the work of Brücke and Helmholtz on vision and art. His interest in drawing and painting lasted all his life, and in recent years he had returned to the study of the relationship between visual physiology and the perception of pictures. *Optics, Painting and Photography* (1970) is concerned mainly with perspective, and how far a painting in perspective can really look convincing to an observer who, instead of viewing it with one eye placed in the ideal position, uses both eyes and moves to different positions. His last publication, in 1975, was on *Vision and Art*.

All Pirenne's scientific work was characterised by acute commonsense and painstaking accuracy. The same qualities appear in his published work. *Vision and the Eye*, which first appeared in 1948 remains the clearest and most readable introduction to the subject. He was internationally recognised as an authority on visual physiology, and in 1972 received an ScD at Cambridge and was appointed a Foreign Member of the Royal Belgian Academy of Sciences.

F. H. C. Marriott

B. B. Roberts

BRIAN BIRLEY ROBERTS, authority on polar problems and ornithologist, died in Cambridge on 9 October 1978 at the age of 65. For thirty years Head of the Polar Regions Section, Foreign and Commonwealth Office, he was also a Research Fellow at the Scott Polar Research Institute, Cambridge and a Fellow of Churchill College.

His interests in the polar regions started when he was still a schoolboy and he became probably the foremost specialist in the world. As an undergraduate at Emmanuel College, Cambridge, he led expeditions to Iceland and East Greenland in 1932 and 1933 and later joined the British Grahamland Expedition (1934-1937). As an ornithologist, he will be particularly

remembered for his paper on Wilson's petrel published in 1940, which has stood the test of time as a good and careful study. It dealt with breeding behaviour, growth and reproductive success; the remarkably long annual migration was illustrated by monthly distribution maps and supported by ringing returns, which proved that individuals return each year to the same mate and burrow in the Antarctic.

In 1940 he took a Cambridge PhD and was awarded the Polar Medal and the Bruce Memorial Prize of the Royal Society of Edinburgh. He received the Back Award of the Royal Geographical Society in 1948. During the war years he served in Naval Intelligence and advised the War Office on cold climate equipment. He revisited the Antarctic in 1950-51, 1960-61, 1964, 1972 and finally in 1976, shortly after his retirement.

Brian Roberts played a formative role in discussions leading to the Antarctic Treaty (1959) which ensured the continuance of the freedom of scientific investigation and the free exchange of results which marked the International Geophysical Year 1957-1958. He did much to promote the Agreed Measures for the Conservation of Antarctic Flora and Fauna (1964) and the Convention for the Conservation of Antarctic Seals (1972). While he asserted that he had had to *eat* more seals than any others present, he was dedicated to conservation and also regarded that convention as the forerunner of a broader convention dealing with other Antarctic marine living resources, especially krill, now being negotiated. Although often the only scientist in a group of diplomats and lawyers, it was to Brian Roberts that they frequently turned for help in resolving a diplomatic impasse.

He played an active role in the formation of the British Antarctic Survey and he had an enduring influence on the development of the library of the Scott Polar Research Institute and the establishment of what developed into the International Glaciological Society. He was a leading expert on Antarctic history, terminology and place names.

He was made CMG in 1969 and in 1976 was awarded the Founder's Medal of the Royal Geographical Society. He was president of the Antarctic Club in 1963 and of the Arctic Club in 1973.

Many senior polar scientists benefited, as young research workers and later, from the stimulation of his presence, his phenomenal range of detailed knowledge and constructive criticism. His beaming smile, kindness, wit, scholarship, experience and great capacity for work will be long remembered by his many friends, who will find it difficult to believe he is no longer there.

R. M. Laws

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